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13. ABSTRACT (Maximum 200 words)

Our primary experimental approach has been to record from single neurons in awake, behaving animals in order to determine the loci of neurons with conditioning-related activity and to quantitate the relationship of this activity to the expression of the conditioned response. Our theoretical approach has been to extend simple computational models of connectionist learning into physiologically plausible neural networks that describe real-time features of conditioned behavior. These network models, and their possible implementations in the brain, suggest experimental tests and provide direction for physiological studies.

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June 30, 1992
Final Technical Report
AFOSR 89-0391 (Adaptive Networks)
Dr. Geneviere Haddad, Ph. D., Program Manager

Biological and Theoretical Studies of Adaptive Networks: The Conditioned Response

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I. Summary

There are two widely recognized reasons why theoretical and biological studies of the conditioned response are importent for adaptive networks research. First, conditioned response learning, or classical conditioning, lies at the conceptual center of connectionist learning. Experimental psychologists who investigate classical conditioning believe their research is about the development of associative 'knowledge structures', and they use any system or preparation that elucidates fundamental mechanisms by which these structures are acquired, maintained, and modified. This line of enquiry has given rise to theoretical models of learning that have been applied to engineering control problems, robotics, decision making, and optimization. Secondly, classical conditioning lies at the heart of neurobiological studies of learning from systems to cellular levels.

One goal of a computational approach to learning is that it provides the ideal instrument for a deep (rigorous) understanding of learning at the functional level while at the same time providing insight into underlying biology. Useful computational models based on biological principles must be evaluated through experimentation with living organisms. Our experimental approach is based on the classically conditioned eye blink/nictitating membrane response (NMR) of the rabbit, which was developed originally by I. Gormezano in the 1960s and has been employed as a model system in scores of laboratories around the world. The conditioned eye blink is a temporally adaptive form of behavior because it anticipates (predicts) the timing of the reinforcing event. Our principal concern are the mechanisms responsible for temporally adaptive conditioned responding. This work enhances the scientific underpinnings of research on adaptive control by the engineering and computer science communities.

Our primary experimental approach has been to record from single neurons in awake, behaving animals in order to determine the loci of neurons with conditioning-related activity and to quantitate the relationship of this activity to the expression of the conditioned response. Our theoretical approach has been to extend simple computational models of connectionist learning into physiologically plausible neural networks that describe real-time features of conditioned behavior. These network models, and their possible implementations in the brain, suggest experimental tests and

provide direction for physiological studies.

Research Objectives

One goal of this project was to investigate how neural systems transform temporal expectations of events into adaptive behavior. The development and expression of temporal expectations is basic to understanding complex movements. Such movements are composed of simple motor responses that are appropriately timed and executed. One ongoing focus of research has been to characterize how information about conditioned stimuli is represented in brainstem structures (e.g., medial geniculates and pontine nuclei) that are afferent to putative learning sites in the cerebellum.

Status of Research

The following is an overview of our research from 1983, the first year of our initial AFOSR grant. This material is taken from my competing renewal proposal for additional support submitted last fall (AFOSR 92-NL-033). (A list of all published accounts of work during this period appears in Section IV.)

- **Reflex pathway** Beginning in the late 1970s, Neil Berthier and I began to investigate the neural circuits mediating the unconditioned NMR. Our starting point was the work of Craig Cegavske, Mike Patterson, and others working with Richard Thompson, showing that the NMR is mediated by the abducens (sixth) nerve. Berthier and Moore (1980) reported that NM extension is caused by eyeball retraction, and that this retraction involves the extraocular muscles, especially the retractor bulbi muscles which are innervated by motoneurons of the accessory abducens nucleus. We went on to characterize the electrophysiology of the reflex pathway (Berthier & Moore 1983). In an appended report, *Muscle activity during unconditioned and conditioned eye blinks in the rabbit*, Berthier shows that the NMR is tightly coupled and highly correlated in time with the activity of extraocular muscles and that this activity, in turn, is highly correlated with the eye blink.

- **Essential circuits for the CR** By 1980 it was clear that telencephalic brain structures (neocortex, hippocampus, basal ganglia, etc) are not essential for learning or generating conditioned eye blinks. That year, John Desmond discovered that a small lesion of the dorsolateral pons completely eliminated the CR while leaving intact the unconditioned reflex (UR) (Desmond & Moore 1982). The critical structure destroyed by such lesions was the supratrigeminal reticular formation, which we later showed projects to the region of the accessory abducens nucleus where these cells could participate in CR output. Shortly thereafter, Thompson's group at Stanford showed that lesions of the cerebellar deep nuclei produced that same pattern of CR disruption and UR sparing observed by Desmond. In fact, most cases of lesions of the supratrigeminal reticular formation that resulted in CR disruption also involved the superior cerebellar peduncle (brachium conjunctivum) and were therefore interpretable in terms of a critical role of the cerebellum.

- **Cerebellar deep nuclei** Chris Yeo and his associates in London performed lesion studies

pinpointing the specific cerebellar nuclei mediating the CR. These regions are the anterior portion of nucleus interpositus (NIA) and the medial aspect (dorsal hump) of the dentate. In retrospect, it has become clear that the essential regions of the cerebellum for CR generation, cortex and deep nuclei alike, are those associated with climbing fiber arising from the dorsal accessory olivary nucleus, the portion of the inferior olive dedicated to sensory-motor control of the eyelids and periocular tissue. The US event, its parameter features, modality, and point of application, determines the specific regions of the cerebellum involved in CR generation. This specificity is possible because of the highly zonal organization of climbing-fiber projections from the inferior olive. By contrast, information about a potential CSs is 'broadcast' throughout neocerebellar cortex by mossy fibers. If synaptic connections are altered in the cerebellum through training, it is the US which instructs the system where these alterations are to be made. These findings refined the preliminary reports from Thompson's lab, and they have been confirmed by other groups. Recording studies support the idea that the motor program for the CR exists in the cerebellar nuclei. Berthier conducted a single-unit study of cerebellar nuclei described in two articles, *Activity of cerebellar deep nuclear cells during classical conditioning of nictitating membrane extension in rabbits* (Berthier & Moore 1990) and *Linear systems analysis of the relationship between firing of deep cerebellar neurons and the classically conditioned nictitating membrane response in rabbits* (Berthier et al 1991). These articles report that the firing of cells in cerebellar nucleus interpositus can be highly predictive of the CR. Anatomical studies of cerebellar nuclei by Marcy Rosenfield confirm previous research in the cat and rat: (a) interpositus cells project to the red nucleus, (b) interpositus cells also project to the contralateral inferior olivary nucleus, (c) the interpositus nucleus receive afferents from cerebellar (HVI) Purkinje cells and from the ipsilateral lateral reticular nucleus, but not from the red nucleus.

- **Cerebellar cortex** Lesion studies by Yeo and his colleagues showed that the production of normal, robust CRs requires the integrity of a small strip of cerebellar cortex associated with innervation of the eye—hemispheral lobule VI of Larsell or HVI for short. Others (Lavond and Steinmetz) have painstakenly confirmed this. Berthier and Moore (1986) reported that HVI Purkinje cells fire in a manner that predicts the CR. The foremost open question is whether Purkinje cells that modulate their activity in relation to CRs are 'trained' by US-triggered climbing fiber input, as would be required by theoretical models of cerebellar learning. In addition to recording studies, Rosenfield has conducted a WGA-HRP study of HVI which confirms previous work in the rabbit by Yeo and his colleagues: HVI receives bilateral mossy-fiber innervation from the pontine nuclei and spinal trigeminal pars oralis. It also receives climbing-fiber innervation from the contralateral inferior olive. The new finding is that HVI also receives a sparse mossy-fiber projection from the contralateral red nucleus. This observation has potentially significant theoretical implications because the red nucleus is a candidate locus of the 'CR Image' posited in the article, *Adaptively timed conditioned responses and the cerebellum: a neural-network approach* (Moore et al 1989).

- **Red nucleus** Red nucleus is a critical structure in the generation of the CR because of its projections to the region of the accessory abducens nucleus, as shown by HRP studies,

and its innervation by the cerebellum via brachium conjunctivum. Furthermore, red nucleus lesions disrupt CRs, as would be expected if the motor program for the CR is routed from the cerebellum to motoneurons via this link (Rosenfield & Moore 1985). Single-unit recordings from red nucleus during CRs revealed the presence of neurons with firing patterns related to the CR production. These data are presented in the article, *Single-unit activity in red nucleus during the classically conditioned rabbit nictitating membrane response* (Desmond & Moore 1991a). The same study, however, suggests that red nucleus could be a site of learning.

- **Computational models** In 1983, we began to develop a real-time version of the Sutton-Barto (SB) model suitable for NMR conditioning (Moore et al 1986). This version of the model has been described in several reports, e.g., the articles *Conditioned stimulus duration in classical trace conditioning: test of a real time model* (Blazis & Moore 1991). In order to address certain limitations of the SB model, Desmond and Moore (1988) developed a neural network model based on the idea that time is represented by delay lines. The Desmond-Moore (DM) model describes real-time aspects of CR topography for both simple and complex training paradigms, such as trace conditioning. The model assumes that CS onset and CS offset engage separate and independent input elements. These input elements are taps off of delay lines that send propagated activity, in two parallel streams, to two processing nodes. One node updates connection weights for CR output; the other node computes the expected times of occurrence of the US. The DM model and its possible implementation in the brainstem and cerebellum are described in the article, *Adaptively timed conditioned responses and the cerebellum, a neural network approach* (Moore et al 1989). Although it accounts for motor output in the context of classical conditioning, the DM model can be regarded as general theory of temporally adaptive prediction and control.

- **Tests of models** Our modeling work has relied on a 'top down' approach. First, we instantiate an algorithmic form of a real-time model and then experimentally investigate its properties in simulation studies. This process has suggested possible plausible implementations of the model in the brain, as illustrated in our 1989 article on the DM model. One prediction of the DM model concerns the response waveforms learned in trace conditioning. The DM model predicts that extending the CS's duration can result in bimodal CR waveforms, with each mode or peak corresponding to the point where the US occurs with respect to CS onset (the first peak) or CS offset (the second peak). These results are described in the article, *Altering the synchrony of stimulus trace processes: tests of a neural-network model* (Desmond & Moore 1991b). Other tests of the model are planned.

Publications: 1983 to Present

Listed here in chronological order are all publications from this laboratory from 1983 to the present, including items that are 'in press' and 'submitted'. It is not an exhaustive list of all publications by the PI or his associates during this period. Support for this work came from the NSF as well as the AFOSR. Copies of the most recent items, not previously forwarded to AFOSR, are appended to this document.

Book

1. Gabriel, M. and Moore, J.W. (Eds.) *Learning and computational neuroscience: Foundations of adaptive networks.* Cambridge, MA: Bradford Books/MIT Press, 1990. 613 pages.

Research Articles

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8. Rosenfield, M.E. and Moore, J.W. Red nucleus lesions impair acquisition of the classically conditioned nictitating membrane response but not eye-to-eye savings or unconditioned response amplitude. *Behavioural Brain Research*, 1985, 17: 77-81.
9. Schmajuk, N.A. and Moore, J.W. Real-time attentional models for classical conditioning and the hippocampus. *Physiological Psychology*, 1985, 13: 278-290.
10. Moore, J.W., Desmond, J.E., Berthier, N.E., Blazis, D.E.J., Sutton, R.S., Barto, A.G. Simulation of the classically conditioned nictitating membrane response by a neuron-like adaptive element: Response topography, neuronal firing, and interstimulus intervals. *Behavioural Brain Research*, 1986, 21: 143-154.
11. Moore, J.W., Desmond, J.E., Berthier, N.E., Blazis, D.E.J., Sutton, R.S., and Barto, A.G. Connectionistic learning in real time: Sutton-Barto adaptive element and classical conditioning of the nictitating menbrane response. *Proceedings of the Seventh Annual Conference of The Cognitive Science Society*, Irvine, California, 1985, 318-322.
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14. Berthier, N.E. and Moore, J.W. Cerebellar Purkinje cell activity related to the classically conditioned nictitating membrane response. *Experimental Brain Research*, 1986, 63: 341-350.

15. Desmond, J.E. and Moore, J. W. Dorsolateral pontine tegmentum and the classically conditioned nictitating membrane response: Analysis of CR-related single-unit activity. *Experimental Brain Research*, 1986, 65: 59-74.

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20. Moore, J.W., Desmond, J.E. and Berthier, N.E. Adaptively timed conditioned responses and the cerebellum: A neural network approach. *Biological Cybernetics*, 1989, 62: 17-28.

21. Berthier, N.E. and Moore, J.W. Activity of deep cerebellar nuclear cells during classical conditioning of nictitating membrane extension in rabbits. *Experimental Brain Research*, 1990, 83: 44-54.

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26. Richards, W.G., Ricciardi, T.N., and Moore, J.W. Activity of spinal trigeminal pars oralis and adjacent reticular formation neurons during the classically conditioned rabbit nictitating membrane response. *Behavioural Brain Research*, 1991, 44: 195-204.

27. Blazis, D.E.J. and Moore, J.W. Conditioned inhibition of the nictitating membrane response in rabbits following hypothalamic and mesencephalic lesions. *Behavioural Brain Research*, 1991, 46, 71-81. **Appended**

28. Berthier, N.E. Muscle activity during unconditioned and conditioned eye blinks in the rabbit. *Behavioural Brain Research*, 1992, 48: 21-28. **Appended**

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1. Moore, J.W. and Solomon, P.R. Forebrain-brainstem interaction: Conditioning and the hippocampus. In Squire, L.R. and Butters, N. (Eds.), *The neuropsychology of memory.* New York: Guilford, 1984, pages 462-472.

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5. Moore, J.W. and Berthier, N.E. Purkinje cell activity and the conditioned nictitating membrane response. In Glickstein, M., Yeo C., and Stein, J. (Eds.), *Cerebellum and neuronal plasticity,* New York: Plenum, 1987, pages 339-352.

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9. Moore, J.W., Berthier, N.E., and Blazis, D.E.J. Classical eye blink conditioning: Brain systems and implementation of a computational model. In Gabriel, M. and Moore, J.W. (Eds.) *Learning and computational neuroscience: Foundations of adaptive networks.* Cambridge, MA: Bradford Books/MIT Press, 1990, pages 359-387.

10. Moore, J.W. Implementing connectionist algorithms for classical conditioning in the brain. In Commons, M.L., Grossberg, S., and Staddon, J.E.R. (Eds.) *Neural network models of conditioning and action.* Hillsdale, NJ: Lawrence Erlbaum Associates, 1991, pages 181-191.

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1. Schmajuk, N.A. and Moore, J.W. *Two Attentional Models of Classical Conditioning: Variations in CS Effectiveness Revisited.* University of Massachusetts at Amherst, Department of Computer and Information Science, Technical Report 87-29, 1987. 33 pages.
2. Blazis, D.E.J. and Moore, J.W. *Simulation of a Classically Conditioned Response: Components of the Input Trace and a Cerebellar Implementation of the Sutton-Barto-Desmond Model.* University of Massachusetts at Amherst, Department of Computer and Information Science, Technical Report 87-74, 1987. 61 pages.
3. Desmond, J.E. *Temporally Adaptive Conditioned Responses: Representations of the Stimulus Trace in Neural Network Models.* University of Massachusetts at Amherst, Department of Computer and Information Science Technical Report 88-20, 1988. 52 pages.
4. Blazis, D.E.J. *Computational and Behavioral Investigations of Real-Time Models of Classical Conditioning.* University of Massachusetts at Amherst, Department of Computer and Information Science Technical Report 90-46. 166 pages.
5. Moore, J.W. *A Mechanism for Timing Conditioned Responses.* University of Massachusetts at Amherst, Department of Computer and Information Science Technical Report 92-3. 12 pages. **Appended**

Neuroscience Abstracts

1. Desmond, J.E., Rosenfield, M.E., and Moore, J.W. Red nucleus and supratrigeminal reticular formation: Brain stem components of the conditioned nictitating membrane response. *Society for Neuroscience Abstracts*, 1983, 9: 331.
2. Moore, J.W., Rosenfield, M.E., and Dovydaitis, A. Brachium conjunctivum and rubrobulbar tract: Brain stem projections of the red nucleus essential for the conditioned nictitating membrane response. *Society of Neuroscience Abstracts*, 1984, 10, 793.
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6. Berthier, N.E. and Moore, J.W. Cerebellar Purkinje cell activity related to the classically conditioned nictitating membrane response. *Society for Neuroscience Abstracts*, 1986, 12: 1418.
7. Desmond, J.E. and Moore, J.W. Red nucleus single-unit activity during the classically conditioned rabbit nictitating membrane response. *Society for Neuroscience Abstracts*, 1987, 13: 841.
8. Blazis, D.E.J. and Moore, J.W. A cerebellar cortical implementation of the Sutton-Barto-Desmond model of the classically conditioned rabbit nictitating membrane response. *Society for Neuroscience Abstracts*, 1987, 13: 842.

9. Berthier, N.E., Barto, A.G., and Moore, J.W. Linear systems analysis of cerebellar deep nuclei cells during performance of classically conditioned eyeblink. *Society for Neuroscience Abstracts*, 1988, 14: 1239.

10. Rosenfield, M.E. and Moore, J.W. Is there a reciprocal connection between red nucleus and interposed cerebellar nuclei in rabbit? *Society for Neuroscience Absracts*, 1988, 14: 493.

11. Moore, J.W., Desmond, J.E., and Berthier, N.E. Adaptively timed conditioned responses and the cerebellum: A neural network approach. *Society for Neuroscience Abstracts*, 1989, 15: 506.

12. Rosenfield, M.E. and Moore, J.W. Reciprocal connections between red nucleus and interposed cerebellar nuclei in rabbit reexamined. *Society for Neuroscience Abstracts*, 1989, 15: 890.

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14. Blazis, D.E.J. and Moore, J.W. Conditioned stimulus duration: Behavioral assessment of a prediction of the Sutton-Barto-Desmond model. *Society for Neuroscience Abstracts*, 1989, 15: 506.

15. Rosenfield, M.E. and Moore, J.W. Red nucleus projections to the accessory abducens nucleus in rabbit reexamined with WGA-HRP. *Society for Neuroscience Abstracts*, 1990, 16: 271.

16. Berthier, N.E. and Moore, J.W. Orbicularis oculi and extraocular muscle activity during unconditioned and conditioned eyeblinks in the rabbit. *Society for Neuroscience Abstracts*, 1990, 16: 916.

17. Rosenfield, M.E. and Moore, J.W. Red nucleus projections to cerebellar cortex (HVI) in rabbit examined with WGA-HRP. *Society of Neuroscience Abstracts*, 1991, 17: 870. **Appended**

18. Hirl, M.J. and Moore, J.W. Single-unit activity in ventrolateral pons during conditioning of the rabbit nictitating membrane response. *Society for Neuroscience Abstracts*, 1992, 18: in press. **Appended**

19. O'Connor, K.N. and Moore, J W. Modulation of activity in the medial geniculate nucleua during auditory trace conditioning. *Society for Neuroscience Abstracts*, 1992, 18: in press. **Appended**

Commentaries and Reviews

1. Moore, J.W. Cerebro-cerebellar learning loops and language. *Behavioral and Brain Sciences*, 1989, 12, 156.

2. Moore. J.W. Computational neuroscience. *Contemporary psychology.* In press. Review of Gluck, M.A. and Rummelhart, D.E. (Eds.) *Neuroscience and connectionist theory*, Hillsdale, NJ: Lawrence Erlbaum Associates, 1990. 405 pages.

Doctoral Dissertations

1. Desmond, J.E. *The Classically Conditioned Nictitating Membrane Response: Analysis of Learning-Related Single Neurons of the Brain Stem*, Ph. D. Dissertation in Biopsychology, University of Massachusetts-Amherst, September 1985.

2. Schmajuk, N.A. *Real-Time Attentional Models for Classical Conditioning and the Hippocampus*, Ph. D. Dissertation in Biopsychology, University of Massachusetts-Amherst, May 1986.

3. Blazis, D.E.J. *Computational and Behavioral Investigations of Real-Time Models of Classical Conditioning*, Ph. D. Dissertation in Neuroscience and Behavior, University of Massachusetts-Amherst, June, 1990.

V. Professional personnel

- John W. Moore, Ph. D. (Psychology, Indiana) Principal Investigator.
- Neil E. Berthier, Ph. D. (Psychology, UMass) Senior postdoctoral associate. Berthier had been associated with the lab for many years. He is presently associated with the department of computer and information science, a position he assumed in August, 1990.
- John E. Desmond, Ph. D. (Psychology, UMass) Senior postdoctoral associate. Desmond had also been associated with the lab for many years. He is presently with EEG Systems Labs, Inc. of San Francisco, a position he assumed in January 1991.
- Iwona Zurawska, Ph. D. (Natural Science, Nencki Institute, Warsaw, Poland) Postdoctoral associate. Zurawska joined the project in July, 1990 and returned to Poland May, 1991 to assume an academic position in physiololgy.
- Kevin O'Connor, Ph. D. (Psychology, Columbia University) Postdoctoral fellow. O'Connor joined the laboratory in July, 1990.
- Marcy E. Rosenfield, B.S. (Zoology, UMass) Departmental Assistant. Rosenfield is a certified AALAS animal care technician.

VI. Interactions (John W. Moore)

Listed here or Professional Interactions during the third and final year of the project. Interactions for Years 1 and 2 are described in their respective annual technical reports.

1. Ad hoc panel member for an NIMH special review, Washington, DC, June 1991.

2. Invited participant NATO ARW on Actic n, Time and Cognition, St. Malo, France, October, 1991

3. Invited lecture, Department of Psychology, University of Connecticut, November, 1991.

4. Society for Neuroscience meetings, New Orleans, November, 1991.

5. Invited participant, New England Conference on Sequencing and Timing, Department of Psychology, University of Massachusetts, January 1992.

6. Invited lecture, Psychobiology Group ('Learning Beer'), Yale University, Febrary 1992.

7. Invited colloquium, Neuroscience and Behavior Program, University of Massachusetts, February 1992.

8. Invited colloquium, Department of Psychology, Northwestern University, April 1992.

9. Adaptive network research seminar, Avionic Laboratory, Wright Research and Development Center, WPAFB, May 1992.

10. Reviewing and Related Activity: Consulting editor for *Psychobiology* and reviewed manuscripts for several journals, including *Behavioural Brain Research*, Psychological Review, *Behavioral Neuroscience*

11. Continuing interactive relationships with A G Barto and other colleagues working in Adaptive Networks: A H Klopf's group at AF Wright Avionics Lab, R S Sutton at GTE Labs, J C Houk and N A Schmajuk of Northwestern University, E J Kehoe of the University of New South Wales, and others.

VII. New Discoveries

Discoveries that might be designated as new were those stemming from experimental research.

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Muscle activity during unconditioned and conditioned eye blinks in the rabbit

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EMGs were recorded from the orbicularis oculi, retractor bulbi and superior rectus muscles in rabbits to investigate the time course of muscle activation during unconditioned and conditioned eye blinks. EMGs from the three muscles showed two responses, with the responses of the orbicularis oculi and retractor bulbi showing the [illegible] latency, and the responses of the superior rectus lagging. The latency of responses to periorbital electrostimulation was about 5 ms, and to air puff stimulation about 10 ms. Results showed a tight coupling of activity between muscles, with cross-correlograms peaking at 0.65 to 0.85 and showing little time shift. Stimulus–response curves showed clear non-linearities in the response of the muscle to changes in stimulus strength. Local anesthesia of the cornea had little effect on unconditionally evoked responses. The form of unconditionally evoked responses was similar with periorbital electrostimulation and air puff stimuli but differed in latency. These results show the form of the eye blink reflex response and will be of importance in interpreting electrophysiological studies of the classically conditioned eye blink of rabbits.

INTRODUCTION

Stimulation of the cornea, periorbital skin, or periorbital hairs of the rabbit results in closure of the eyelids, retraction of the globe, and extension of the nictitating membrane (NM). These movements are caused by contraction of the orbicularis oculi (OO), which is internal to the eyelids and causes eyelid closure, and by contractions of the retractor bulbi (RB), recti and inferior oblique extraocular muscles that cause retraction of the globe[2,6,8]. Extension of the NM in rabbit passively results from retraction of the globe (e.g. ref. 2). Contractions of other muscles of the head and neck frequently accompany defensive eye blinks.

The abducens, accessory abducens, facial, and oculomotor nuclei contain the motoneurons that are responsible for the defensive eye blink. The motoneurons that control the OO are located in the facial nucleus and those that control the RB during defensive blinks are located in the accessory abducens nucleus (e.g. refs. 3,5). The recti and oblique muscles that contribute to defensive blinking are innervated by the oculomotor and abducens nuclei[2,6,8].

The rabbit eye blink is used as a model system in investigations of the physiological basis of classical conditioning. These studies usually record the position of the NM as an index of the unconditioned (UR) and conditioned responses (CRs). Single-unit recording studies have correlated the activity of central neurons with the occurrence and performance of the CR, but neural activity is usually only compared to NM position (e.g. ref. 4). This procedure may lead to overlooking some eye blink neurons that are not correlated with NM position, and may lead to labeling neurons as projecting to the musculature controlling the NM when in fact they project to the external lid or oculomotor musculature. Currently, little data is available on the time-course of activation of the various muscles underlying the blink in rabbit. Papers on cat[10,18,19], and rabbit[13] present recordings from either the OO or RB. Woody and Engel[20] compared activity of the levator oris and OO during blinks elicited by glabella tap in cats. McCormick et al.[14] recorded OO EMG concurrently with NM position and found that OO activity was only moderately correlated with NM position. It seems likely

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that a higher correlation would have resulted in McCormick et al. if OO and RB EMGs were compared directly.

The primary purposes of the present paper are to investigate the time-course of muscle activation during conditioned and unconditioned blinks and to determine how the activity of the eye blink musculature is interrelated. To achieve these aims NM, RB, superior rectus (SR) EMGs, and NM and lid positions were recorded during unconditionally elicited blinks as well as during classical conditioning sessions. The SR was chosen because it is likely that it is representative of the extraocular recti muscles. The effects of changing the strength, duration, and modality of the eliciting stimuli were investigated. Because of the technical difficulties in measuring the positions of the external lid and NM during rapid movements such as blinks, this paper does not specifically address the details of the transformation of muscle activity to lid and NM movement.

MATERIALS AND METHODS

EMGs from the OO, RB, and SR were obtained from six New Zealand albino rabbits during classical conditioning sessions and during unconditioned blinks. NM and lid positions were recorded with minitorque potentiometers along with EMG recordings. Three rabbits were prepared for chronic recording and three rabbits were used as acute preparations.

Implantations of EMG electrodes were done in generally anesthetized animals. Chronic animals were anesthetized with a mixture of ketamine (35 mg/kg), acepromazine (0.3 mg/kg), and xylazine (5 mg/kg), and acute animals were anesthetized with sodium pentothal (to effect). Xylocaine was injected into wound margins, subconjunctively, and on the cornea after induction of general anesthesia. Single-stranded Teflon-coated stainless steel wires (0.003 inch bare; 0.0045 inch coated) were used as EMG electrodes. Each electrode was prepared by placing a length of wire in a 27-gauge hypodermic needle, stripping 1 mm of insulation from the end of the wire, and bending the wire to form a hook. The wire was then pulled back through the needle until only the hook was exposed. The wire was then placed into the muscle and the needle removed. A pair of wires was placed into each of the muscles. All animals had EMG electrodes placed in the OO, RB, and SR muscles. In chronic animals wires were run subcutaneously to a connector that was fixed to the top of the head with dental cement. In acute animals the wires were run subcutaneously to a location on the top of the head and then attached directly to the input stage of a preamplifier. During surgery a bolt was also attached to the skull so that the head could be rigidly held during the recording sessions[4]. Animals were then allowed to recover from the general anesthetic.

NM position was measured by a potentiometer that was rigidly connected to the NM[4]. Eyelid position was measured by a potentiometer that was rigidly connected to the upper eyelid. The rigid connection from the lid and NM to the potentiometers was fashioned from lengths of 30-gauge stainless steel tubing. The tubing was connected to the lid and NM via a short loop of 4–0 nylon suture. Because the primary purpose of this investigation was the recording of EMGs and because of mechanical constraints of the apparatus, the potentiometers could not be placed optimally. This led to smaller potentiometer rotations and smaller voltage signals from lid and NM movements than is normal for behavioral experiments. These smaller potentiometer signals were also less reliable than usual and did not always accurately reflect lid and NM movements. On many occasions, the potentiometer signals did appear to accurately reflect the latency and amplitude of NM and lid movement when compared to blinks measured in other studies using conventional methods in the same laboratory (e.g. ref. 4).

EMG activity was amplified, band-passed at 10–3,000 Hz, displayed on an oscilloscope, and recorded on a tape recorder for off-line analysis. EMG activity was also full-wave rectified and integrated with a time constant of 7.5 ms. For off-line analysis, integrated EMG and position data were 8-bit A/D converted at 200 Hz. Data were stored on disk and displayed on a graphics terminal. Cross-correlograms were computed between the rectified, integrated EMGs with the cross-correlation at time-shift j equalling[12]:

$$r(j) = \frac{\sum [x_{t-j} - \bar{x}]\,[y_t - \bar{y}]}{\sum [x_{t-j} - \bar{x}]^2 \sum [y_t - \bar{y}]^2},$$

where x and y are the sequences of samples from the integrated EMGs of two different muscles, $\bar{x}$ and $\bar{y}$ are the means of the two samples, and j is the time shift. Time shifts were computed from −100 to 100 ms in steps of 5 ms.

Unconditioned stimuli used to elicit eye blinks were electrical stimulation of the periorbital skin and air puffs. Electrostimulation of the periorbital skin was performed as in conditioning experiments (e.g. ref. 4). Briefly, two 9-mm wound clips were placed in the skin at the temporal canthus and just inferior to the eye. Electrical stimuli delivered to the periorbital skin were generated by a Grass S88 stimulator and isolated with

a Grass PSIU 5 stimulus isolation and constant current unit.

A cylinder of compressed nitrogen was used as a source for the air puffs. The gas was regulated at the tank (1–20 psi) and delivered in pulses of 30–300 ms duration. Pulse duration was controlled by a solenoid. The exit port of the valve (3 mm in diameter) was directed at the cornea and placed 1–2 cm from the cornea. A miniature microphone was placed just above the eye to measure when the puff reached the cornea. The exit port–cornea distance was the same as the exit port–microphone distance. The output of the microphone also provided a measure of the duration of the puff.

Classical conditioning trials were given with a 1,200 Hz tone as the CS, and electrical stimulation of the periorbital skin as the US. The interstimulus interval was 350 ms for some animals and 500 ms for others. The CS and the US co-terminated. The US consisted of a series of two 0.1 ms duration pulses of electrostimulation at an interpulse interval of 7 ms. The intertrial interval was 30 s. Details of classical conditioning procedures can be found in ref. 9.

RESULTS

Recordings from chronic and acute animals were very similar in latency and form. The data on which the Figures of the current paper are based are taken from acute recordings because the signal-to-noise ratio and reliability of the EMGs were generally higher in the acute animals.

Unconditioned response

Eye blinks elicited by air puffs and periorbital electrostimulation were of different latency but otherwise similar in time-course. Fig. 1 shows typical examples of the OO, RB, and SR EMG responses to air puff (Panel A) and periorbital electrostimulation (Panel B). Panel A shows air puff-elicited responses, and Panel B shows electrostimulation-elicited responses. The stimulus artifact with air puff stimuli, unlike electrostimulation stimuli, was usually too small to be noticed in recordings of normal gain and did not contaminate integrated EMGs. The stimulus artifact of periorbital electrostimulation did lead to significant contamination of the EMG, but neural responses could be separated from artifact by comparing recordings with opposite stimulus polarity. OO and RB EMG responses were always much greater in amplitude than SR EMG responses. Examination of EMGs revealed short-latency and long-latency responses to the eliciting stimuli in each of the muscles. These responses are usually called the early response (R1) and late response (R2) (e.g. ref. 7). In Fig. 1 R1 is indicated by the asterisk and R2 by the hatched bar.

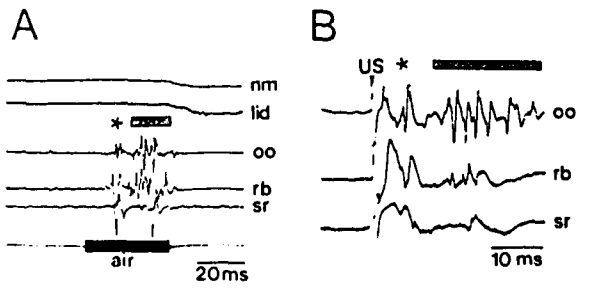

Fig. 1. RB, SR, and OO EMG responses to periorbital stimulation. The responses are raw and not integrated EMGs. R1 is indicated by the asterisk and R2 is indicated by the hatched bar A: EMG responses to air puff stimulation. The timing of the air puff is shown by the lower tract. B: EMG response to electrostimulation of the periorbital skin. Onset of the stimulus is indicated by the arrow.

Latencies of R1 and R2 were measured from photographs or directly from the oscilloscope. The latency of the R1 was very reliable once the eliciting stimulus was significantly above threshold, while the latency of R2 was harder to measure and seemed more variable because of the unsynchronized nature of the motor unit activity. The latencies of the responses did vary with the type of eliciting stimulus. Periorbital electrostimulation resulted in shorter latency EMG responses than air puffs. Air puffs elicited R1 and R2 responses at about 10 and 15 ms latency (e.g. Fig. 1A), while electrostimulation produced responses at about 5 and 10 ms latency (e.g. Fig. 1B). Regardless of the eliciting stimulus, the R1 response appeared to be a relatively synchronous volley, while the R2 response appeared to be the result of a longer period of unsynchronized activity. In a given animal, the RB and OO EMGs responses became activated at slightly different times, with neither muscle being consistently activated before the other across animals. The difference in time between the activation of the RB and OO was always less than 1 ms. The SR response always lagged behind the RB and OO responses.

Effects of stimulus amplitude and duration

In order to investigate how the unconditioned reflex transforms the eliciting stimulus into an eye blink, air puff stimuli of differing amplitude and duration were presented to three animals. Air puffs were used to avoid the electrical artifacts introduced by electrostimulation. Each combination of intensity and duration was given 10 times and the resulting rectified EMG responses for each combination were averaged together. Fig. 2 shows the results for one of the animals that are representative of the three animals. Panel A shows responses to an 8

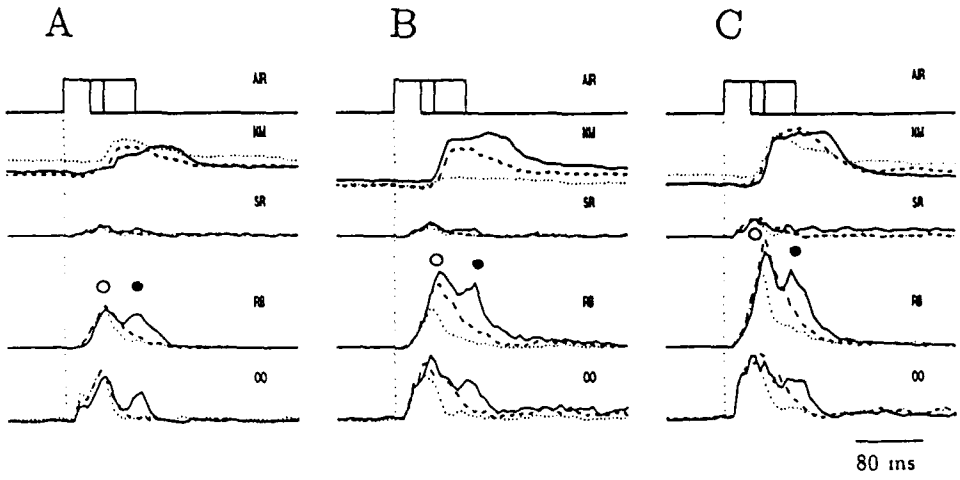

Fig. 2. Average responses to 8 (A), 12 (B), and 16 (C) PSI air puffs. The traces from top to bottom show the time course of the stimulus, NM position, and SR, RB, and OO rectified EMG activity. The dotted traces are the responses to 30-ms stimuli, the dashed traces responses to 45-ms stimuli, and the solid traces the responses to 80-ms stimuli. The onset of the air puff is indicated by the vertical dotted line. The combined R1, R2 response is indicated by the open circle, and the long-latency response is indicated by the filled circle.

PSI stimulus, Panel B to a 12 PSI stimulus, and Panel C to a 16 PSI stimulus. Stimulus durations of 30, 45 and 80 ms are shown in each of the panels.

Fig. 2 shows that the NM response lengthened with stimulus duration, with stimuli of 80 ms duration resulting in a long, step-like response. NM position required 2–8 s to return to baseline after termination of the stimulus. EMG activity was at a very low level during the decay of NM extension.

The R1 and R2 responses are not easily separated in Fig. 2 because the EMGs are rectified, integrated, and averaged. The combined R1, R2 complex is indicated by the open circle. A long-latency EMG volley, with a latency of about 70 ms, is visible in the data from the 80-ms duration stimuli (filled circle). The long-latency EMG volley was not the R2 volley described above, as the latency of the long-latency volley was significantly longer than the R2 response in the EMG. Stimulus durations of 30 and 45 ms did not elicit the long-latency volley, whereas stimulus durations of greater than 80 ms invariably elicited a long-latency volley. Examination of data from other animals indicated that a stimulus duration of 50 ms was sufficient to elicit the long-latency volley. In Fig. 2 the long-latency response is seen in all three panels and in all three muscles when stimulus duration was 80 ms.

Fig. 2 also shows that there was a rapid transition from small to large amplitude NM extensions when stimulus strength was increased. With all stimulus durations shown in Panel A and with the shortest duration stimulus of Panel B, NM extension is small, whereas in Panel C and in Panel B's two other traces, NM extension is large.

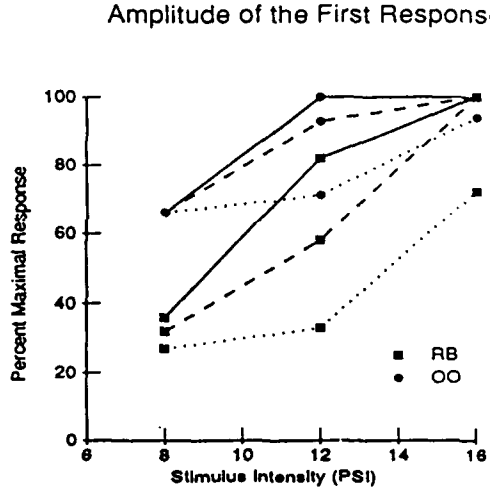

Fig. 3. Amplitude of the first response in the RB and OO muscles to air puff stimuli of 8, 12, and 16 PSI. The dotted lines are the response to 30-ms stimuli, the dashed lines are the response to 45-ms stimuli, and the solid lines are the response to 80-ms stimuli. The amplitude is scaled so that the response to the strongest stimulus is 100%.

Fig. 3 shows the amplitude of the initial EMG response (i.e. the one indicated by the open circle in Fig. 2) as a function of stimulus intensity and duration for a single representative animal. Each data point shows the average amplitude of the initial EMG peak from a series of 10 presentations. The responses of RB for stimuli of varying intensity and duration are shown by the filled squares. Only the RB responses to the 45-ms duration stimuli resemble a linear stimulus–response curve. The other responses seem significantly non-linear; changes in response intensity when the sti-

mulus was weak only had small effects on response amplitude, changes of stimulus intensity with a strong stimulus similarly had small effects on stimulus amplitude. When the stimulus was of moderate intensity, changes in intensity had a large effect on response amplitude.

This same general pattern shown by the RB was repeated for the stimulus–response curve of the OO. The activity of the SR was an order of magnitude less and changes in the size SR EMG were small when the amplitude or duration of the eliciting stimulus was varied.

Experiments where the strength of the stimulus was lowered showed that threshold intensities for EMG responses in OO and RB were identical. However, Fig. 3 shows that activity in OO was closer to maximal at low intensities than RB. With the 8 PSI stimulus, RB responded with an amplitude that was 28–36% maximal, the OO at 66% maximal. This illustrates that OO activity recruited earlier and saturated at a lower stimulus level than RB.

Effect of head tilt on EMG activity

In order to confirm that the low amplitude SR recordings were indeed from the SR and not a neighboring muscle, and to investigate how the EMG blink responses change with different starting positions of the globe, we recorded EMGs during blinks with the head of the animal fixed at different angles of roll. At different angles of roll, the SR was tonically activated to maintain the visual streak of the retina in line with the horizon. Fig. 4 shows the EMGs in a typical experiment where the animal was rolled to 20° from horizontal. Panel A shows the air puff evoked EMGs when the head was level, and Panel B shows the evoked EMGs

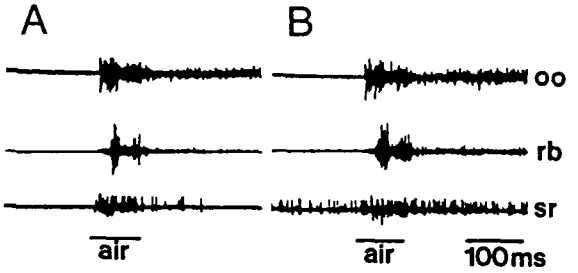

Fig. 4. Effect of head tilt on eye blink EMGs. The left panel shows activity with the head level, and the right shows activity with the head at 20° of roll.

when the head was rolled. Fig. 4B shows that SR EMG was tonically active when the head was tilted.

A small increase in RB tonic activity could also be discerned when the head was rolled (not shown). This was expected sine the RB electrodes were in the dorsal area of the muscle and its activity would presumably increase with globe rotation. These results show that tonic SR activity could be recorded when expected, and that the tonically active SR motor units were similar in amplitude to the motor units that responded to air puff stimulation. It is likely, that the SR EMG activity recorded to blink eliciting stimuli was the result of SR activity and not the result of activity of neighboring muscles.

Cross-correlations between muscles

In three different animals, a series of stimuli were presented of varying duration and intensity. The goal was to elicit a series of eye blinks of a variety of amplitudes and durations. Cross-correlations were then computed between the OO and RB, OO and SR, and RB and SR rectified, integrated EMGs. Fig. 5 shows these correlations for a series of air puff presentations. The

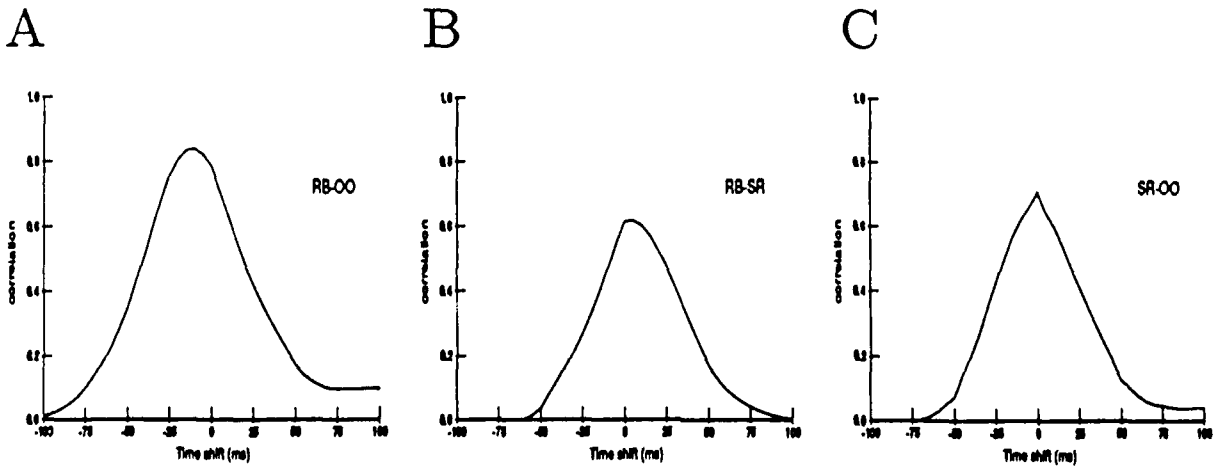

Fig. 5. Cross-correlograms of RB, OO, and SR muscle activity to air puffs. From left to right are RB-OO, RB-SR, and SR-OO cross-correlograms. The responses shown are averages of data from 10 trials.

peak correlation for Panel A was 0.82, Panel B 0.62, and Panel C 0.69. The greatest peak shift of a cross-correlogram from the three animals was 10 ms.

These results indicate that the integrated EMG of the three muscles was highly correlated, with minimal time shift between muscles. The high correlations seen in the cross-correlograms of integrated EMGs seem primarily to reflect similarity in the peaks, onsets, and shapes of the EMGs. Examination of the raw data indicated two reasons why the peak correlations were not higher. The first is that the OO showed spontaneous activity and a much slower return to baseline, while the RB and SR showed little spontaneous activity and a rapid return to baseline. The second reason is that SR activity was significantly lower in amplitude, and manipulations of stimulus intensity and duration had less effect on the SR EMG than the OO and RB EMGs (cf. Fig. 1).

Effect of Xylocaine to the cornea

In order to determine the contribution of corneal afferents to the unconditioned reflex we examined US-evoked EMGs before and after saturation of the cornea with 1% Xylocaine. Adequate anesthesia of the cornea was confirmed by the lack of reflex responses to touches of the cornea with a probe. The present results did not depend on whether air puff or electrostimulation of the periorbital skin was used as the eliciting stimulus. Fig. 6 shows data from air puff elicited blinks because of the lack of stimulus artifact with the air puff. The responses of the RB, OO, and SR muscles, as well as the NM position, before and after local anesthesia of the cornea are shown. Ten stimulus presentations were given followed by saturation of the cornea with Xylocaine. After waiting 5–10 minutes, ten stimulus presentations were given of the same amplitude and duration. Fig. 6 shows the averaged integrated EMGs from each series of presentations. As can be seen, the eye blink EMGs were little effected by the Xylocaine.

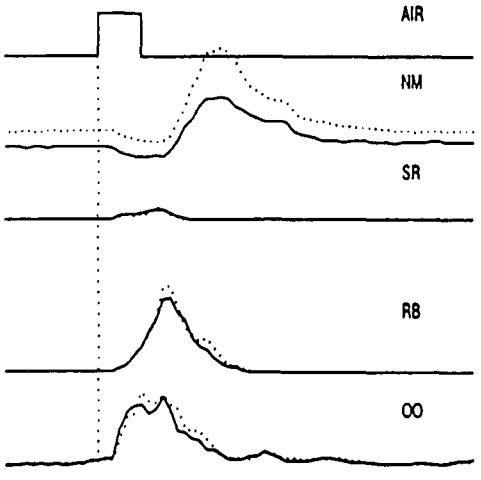

Fig. 6. Eye blink before and after application of Xylocaine to the cornea. The solid trace shows th response before and the dotted trace the response after Xylocaine application. The responses shown are averages of data from 10 trials. The air puff was 30 ms in duration and its application did not cause a visible stimulus artifact in the integrated EMGs.

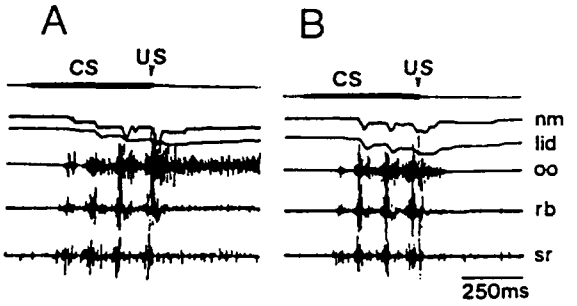

Fig. 7. Multiphasic CRs during conditioning. Recording from two trials are shown. From top to bottom: CS and US timing, NM position, lid position, OO, RB, SR EMG records. Note the high correlation of activity across muscles.

EMG activity during conditioning

EMG activity of the OO, RB, and SR was highly cross-correlated during conditioning sessions. Fig. 7 shows EMGs recorded during a conditioning trial. These CRs are atypical in that they were multi-peaked, but they illustrate the tight coupling of EMG activity between muscles. Examination of the EMGs shows that a burst in one muscle was invariably accompanied by bursts in other muscles. Most of the EMG bursts were followed by inflections in the NM position trace.

Cross-correlograms were computed for the EMGs obtained during conditioning sessions. Fig. 8 shows three correlograms for OO-RB, OO-SR, RB-SR muscles. The correlograms typically peaked at 0.65–0.85 with time shifts of less than 10 ms.

DISCUSSION

The results of this experiment show that the SR, RB, and OO are all involved in the rabbit eye blink. EMG activity in the OO and RB was very similar in threshold, timing and amplitude, while EMG activity in the SR was an order of magnitude smaller. As shown by earlier investigators[11,16], the latency of R1 is very reliable. The activity of the three muscles was highly correlated during both conditioned and unconditioned blinks. The finding that EMG activity was highly correlated during blinks was somewhat surprising because the muscles control two systems, the eyelid and the globe, that are not directly coupled. One might expect that a coordinated blink would require the globe to be retracted

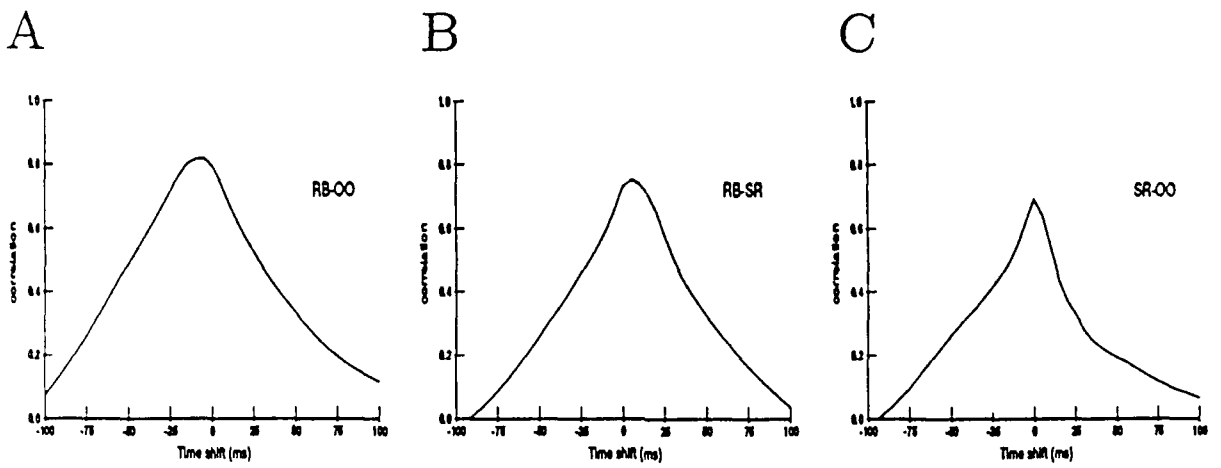

Fig. 8. Cross-correlograms of RB, OO, and SR muscle activity during classical conditioning. The panels from left to right show RB-OO, RB-SR, and SR-OO cross-correlograms.

before the closure of the lids (or vice versa). A second factor that would degrade the cross-correlations between muscles is the fact that the biophysical properties of the blink muscles differ significantly. For example, Steinacker and Bach-y-Rita[17] showed that while the recti and oblique muscles contain both fast twitch and tonic fibers, the RB contains mainly a single intermediate speed fiber type. Consistent with the lack of RB tonic fibers was the result that the OO showed more tonic activity than the RB and SR.

The results of the present study and others provide a good estimate of the time sequence of activity during the blink reflex in cats and rabbits. One complicating factor is the eliciting stimulus: studies using air puff from different laboratories show an inconsistency of latencies primarily because of the difficulties in controlling and assessing the air puff. Studies using periorbital electrostimulation or electrical stimulation of the trigeminal system show more consistent timing across laboratories. Berthier and Moore[3] showed that RB motoneurons respond at about 4.5 ms latency to periorbital electrostimulation. The R1 latency in the present paper was about 5 ms, and in cats about 5 ms[10,18]. Quinn et al.[15] recorded the shortest latency globe retractions at 9.3 ms. Stimulation of the trigeminal nerve near its entrance to the brainstem causes an eye retraction with 5.3 ms latency[3]. These figures from different studies involving cats and rabbits are consistent within about a millisecond, and show that the reflex is very rapid with the bulk of the time required to convert the EMG activity to movement of the globe (about 4–5 ms). The timing for electrical trigeminal stimulation extrapolates to air puff stimulation if one assumes that significant time is required for the air puff to cause discharge of trigeminal sensory neurons.

The current results show that the blink is the result of coordinated action of several muscles. The UR is very reliable and consistent from trial to trial when the duration and amplitude of the eliciting stimulus is constant. Transmission in the UR pathway is subject to modulation as shown by reflex modulation experiments (e.g. ref. 7). The high degree of coupling of the blink muscles observed in the present experiment suggests that a single neural center may be responsible for generating the reflex response. However, the present results do not rule out the possibility that the tight coupling may be the result of parallel activity in several pathways that is synchronized by the US.

The response of the eye blink muscles to stimuli of various intensities and durations showed pronounced non-linearities. At low or high levels of stimulation, changes in stimulus intensity or duration had little effect on EMG responses. At moderate levels of stimulation, changes in stimulus strength resulted in large changes in the EMG response. This type of stimulus–response function suggests that there is an intermediate value of stimulus strength below which EMG responses are small, but above which EMG responses are large or 'full-blown'. The stimulus–response functions for the RB and OO were very similar, but the OO reached a maximal amplitude at a lower stimulus strength than the RB. Underlying the narrow region of the stimulus–response function where amplitude modulation is possible may be the high innervation ratio of the muscles used for the blink. For example, Steinacker and Bach-y-Rita[17] showed that the innervation ratio of the RB was 50 to 1, a value that would be incompatible with fine control of muscle tension.

The blink response seems to be primarily initiated by periorbital skin and hair receptors, and not by corneal afferents, because anesthesia of the corneal receptors had little noticeable effect on the amplitude or time-

course of the elicited blink. In humans, Rushworth[16] showed, and Beradelli et al.[1] confirmed, that corneal stimulation alone elicits an EMG response at a latency of 30–40 ms, a result that strongly suggests that corneal afferents are not involved in R1. One limitation of the current study is that the corneal anesthesia experiment was not performed while systematically varying the stimulus magnitude and duration. It is possible that corneal anesthesia might have a discernable effect at a particular stimulus strength. These observations do not preclude a significant role for corneal receptors in modulating the blink, particularly during conditioning where a strong nociceptive input from corneal receptors may enhance conditioning. In fact, preliminary data from this laboratory suggests that eliciting a strong EMG response in the blink musculature is not always sufficient to support conditioning.

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Conditioned inhibition of the nictitating membrane response in rabbits following hypothalamic and mesencephalic lesions

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Rabbits were trained on a Pavlovian conditioned inhibition (CI) task using light as the reinforced conditioned stimulus (CS +) and the same light compounded with a tone as the nonreinforced CS – . The conditioned response was the nictitating membrane response. After attaining a criterion of CI performance, animals received radio-frequency lesions of the hypothalamus (n = 11) or midbrain (n = 14). For the hypothalamic lesion cases, primary damage extended from the optic chiasm to the pretectal region. For the mesencephalic lesion cases, primary damage ranged from the most rostral portions of the periaqueductal grey (PAG) caudally to the tegmental reticular formation at the level of the third nerve. Prior research suggested that the hypothalamic lesions would disrupt retention of CI by increasing responding to the CS – . Except where a lesion impinged upon the zona incerta, no CI disruption was observed. In accordance with previous studies (Berthier, N.E. and Moore, J.W., *Physiol. Behav.*, 25 (1980) 667–673; Mis, F.W., *J. Comp. Physiol. Psychol.*, 91 (1977) 975–988), post-lesioning CI disruption was observed in some of the mesencephalic lesion cases involving the posterior commissure, PAG and/or accessory oculomotor nuclei. However, CI performance recovered over the course of retraining.

INTRODUCTION

Pavlovian conditioned inhibition (CI) has proven to be a powerful tool in the investigation of response suppression in learning and memory[1,9]. In CI training, an animal is presented with a conditioned stimulus (CS +), for example, a light (L), that is always paired with an unconditioned stimulus (US). On other trials, L is presented together with another CS, for example, a tone (T), that is the conditioned inhibitor. The LT compound (CS –) is never paired with the US. Animals typically learn to perform conditioned responses (CRs) to L and to suppress CRs to the LT compound. Unlike simpler forms of differential conditioning, the CI paradigm requires that the animal attends fully to the stimuli presented. Differential conditioned responding would not be possible otherwise. A brain lesion that selectively disrupts CI would manifest itself as an elevated level of conditioned responding during CS – trials with no loss of responding to CS + .

The rabbit nictitating membrane response (NMR) preparation has been useful in the investigation of CI[1,2,17,18,20,21,23,28,33], but, despite recent gains in understanding the neural substrates of the conditioned NMR[12,32], lesion studies have not yet indicated the neural structures essential for the expression of CI in this preparation. Although the frontal neocortex was once regarded as essential for learned inhibition[3,4,9], decorticated rabbits can acquire and perform CI[23]. Furthermore, if rabbits acquire CI and then undergo decortication, they reacquire CI faster than controls[33]. Notably, decorticates showed an initial impairment of CI retention, when compared to sham-operated controls, but this initial disruption was transitory. Related studies have failed to implicate the hippocampal formation in CI of the conditioned NMR[28]. Lesions of certain midbrain structures, however, have been reported to interfere with CI[1,20].

Berthier and Moore[1] observed disruption of previously acquired CI of the NMR following lesions of the periaqueductal grey (PAG) and the pretectum-posterior commissure region. Degenerating fibers resulting from

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these lesions (Fink–Heimer method) projected to the posterior hypothalamus, a region known to support self-stimulation in rabbits[15]. Thus, lesions in the critical regions might have affected the normal functioning of brainstem reward circuits[5]. This is not an unreasonable idea because a conditioned inhibitor in a defensive conditioning task, such as the NMR preparation, reliably signals the nonoccurrence of the (presumably aversive) US[7]. Accordingly, the present study assessed the effects of posterior hypothalamic lesions on CI performance.

In addition, we reexamined CI performance following lesions of mesencephalic regions described by Berthier and Moore[1] and Mis[20]. Unlike the former study[1], however, in which animals were sacrificed after three daily sessions of CI retraining, we allowed enough post-operative retraining for recovery of preoperatively established CI to manifest itself. We also assessed pre- and postoperative CI in both eyes in this group of animals. Examination of the performance of both eyes allowed assessment of possible effects of contralateral damage and general arousal or stress.

METHODS

Animals

Forty-one mature New Zealand albino rabbits, obtained from a licensed local supplier, were used in this study. They were housed individually under 24 h/dim light with free access to food and water. Their weights ranged from 2.0–3.3 kg at the time of surgery. Sixteen of the 41 animals were dropped from the study for various reasons: (a) six failed to attain the preoperative training criterion (see below); (b) three died during the postoperative period; (c) three sustained red nucleus damage that interfered with their ability to make CRs[25]; (d) in four cases, lesions failed to include structures of interest in this study. (There was no evidence of CI interference in these cases). Of the remaining 25 animals, Cases 1–11 sustained hypothalamic lesions. Cases 12–25 sustained mesencephalic lesions. The hypothalamic group received CI training of the right eye, as described below. The mesencephalic group was trained and tested in both eyes.

Apparatus

Four rabbits were run concurrently in a four-drawer, sound-attenuated, and ventilated file cabinet. The apparatus was essentially the same as those used in previous studies from this laboratory[1,2,17,18,20,33]. Each animal was confined to a Plexiglas rabbit restrainer[11] and fitted with a minitorque potentiometer (Conrac 85153) that had been affixed to a headset. The potentiometer was coupled to the nictitating membrane via a loop of suture. NMR movements were then recorded as DC voltage changes by a Grass Model 5D polygraph. A CR was defined as a positive pen deflection of at least 1 mm within the CS–US interval, a deflection corresponding to an NMR of 0.5 mm.

The CS + during CI training was a light (hereafter denoted L +) delivered by a single 6 V incandescent lamp located behind a translucent panel approximately 10 cm in front of the animal's head. The CS – consisted of the light CS and a concurrent tone (1200 Hz, 85 dB SPL). CS – trials are hereafter denoted LT – . The duration of the CS on both types of trials was 550 ms. On L + trials, the light occurred with the US, a 1.5 mA, 60 Hz shock, at an interstimulus interval of 500 ms. The US was delivered across two 9 mm Clay–Adams wound clips affixed to the inferior and posterior margins of the eye.

CI training

Prior to the initial training session, a suture was placed in the nictitating membrane for attachment to the recording potentiometer[22]. The wound clips for delivery of the US were crimped into the periocular marginal tissue. The animals were restrained and habituated to the apparatus for 20 min before training commenced.

Each training session consisted of 50 L + and 50 LT – trials arranged pseudorandomly so that no more than two trials of the same type occurred consecutively. The intertrial interval was 15 s. Daily sessions were given until each subject met the criterion of at least 90% CRs to L + , and less than 30% CRs to LT – . Cases 1–11 were trained on the right eye only, i.e. the US was applied to the right eye, and this was the eye from which NMRs were recorded, both pre- and postoperatively. Cases 12–25 were trained on the left eye to criterion after attaining criterion on the right eye. During postoperative testing, however, animals in this group received 50 trials on one eye (25 L + and 25 LT –) and 50 trials on the other eye during each daily session, the order alternating from one day to the next.

Surgical procedures

Following attainment of the CI criterion, animals 1–11 received lesions to the posterior hypothalamic region. Animals 12–25 received lesions of the mesencephalon. Animals were anaesthetized by injection of chlorpromazine (4 mg/kg, i.m.) followed by sodium pentobarbital (22 mg/kg, i.v.) and Xylocaine (1%, at points of incision). Using standard stereotaxic techniques, the lesioning electrode was positioned accord-

g to coordinates obtained from Sawyer et al.[27]. The sioning electrode consisted of a 00 stainless-steel sect pin, insulated with enamel except at the tip. A idio-frequency lesion (Grass Instruments Model M4) was produced by passing a current through the ectrode for 30 s, increasing from 0 to 20 mA over that me period.

Following Berthier and Moore[1], animals were allow- l three days for recovery. This relatively brief recovery eriod mitigated secondary degeneration effects. Rec- very was followed by CI testing, which consisted of -7 daily CI retraining sessions. At the conclusion of sting, animals were then overdosed with sodium entobarbital and perfused intracardially with 0.9% line followed by 10% formalin. Brains were removed, ored in formalin for 3 days and then stored in sucrose r 3–4 days. Coronal 60 μm sections were made rough the lesioned regions, and every third section as mounted and stained with 0.5% Cresyl violet.

nalysis of CI performance

A CI disruption index (*DI*) was computed for each nimal using the formula described in Berthier and loore[1]. The *DI* was computed as follows:

$$DI = \frac{(\%\ \text{C Rs to L}+_{pre} - \%\ \text{C Rs to LT}-_{pre}) - (\%\ \text{C Rs to L}+_{post} - \%\ \text{C Rs to LT}-_{post})}{(\%\ \text{C Rs to L}+_{pre} - \%\ \text{C Rs to LT}-_{pre})} \quad (1)$$

here pre refers to the rate of responding to the given imulus on the last training session before lesioning, nd post refers to the rate of responding on the first aining session after lesioning. Disruption of CI is indi- ted by a positive value of *DI*; improvement of CI erformance is indicated by a negative value of *DI*; and o change in performance is indicated by a zero value *DI*. Note that animals subjected to mesencephalic sions were trained preoperatively and postoperatively n both eyes. Hence, each of these animals contributed wo *DI*s.

Two criteria, both involving the *DI*, were used to esignate an animal's CI performance. Animals with a *I* greater than the mean *DI* for their group (e.g. ypothalamic or mesencephalic) met one criterion for sruption. A second criterion was that training be dis- pted with respect to responding to LT −, because *DI*s n be increased as a consequence of impaired respond- g to L +.

RESULTS

Lesion reconstructions

Figs. 1–4 are reconstructions of lesions from the 25 animals that completed the study. Representative coronal sections are numbered by their rostral distance in millimeters from the level of the accessory abducens in histologically prepared tissue. The column of sections for each animal runs rostral to caudal from top to bottom, and the animal's right appears as the reader's right.

Behavioral data were excluded from subsequent analyses for Cases 1 and 2 as these subjects received lesions of the optic chiasm (Fig. 1) that resulted in lowered responding to the light CS +.

CI performance: hypothalamic lesions

Cases 3–11 received hypothalamic lesions following preoperative CI training to the right eye. Table I indicates that animals required a mean of 5.56 (S.E. = 0.75) preoperative CI training sessions to attain criterion. The grand mean for the *DI*s was 0.30 (S.E. = 0.10). None of these animals showed disrupted performance of CI; in all but one case, the increase in *DI* was due

TABLE I

Animals with hypothalamic lesions

CRT, number of sessions required for the CI criterion of attaining at least 90% responding to L + and at most 30% responding to LT −. Pre and post designations refer to before and after surgery, respectively. LH, lateral hypothalamus; PH, posterior hypothalamus; ZI, zona incerta.

Case	*DI*	*CRT Pre*	*Primary lesion*			*CRT Post*
			LH	*PH*	*ZI*	
11	0.73*	5		X	X	6
9	0.72	10		X	X	4
4	0.49	6	X			2
10	0.46	8		X	X	3
5	0.17	5	X			2
3	0.13	5	X			2
7	0.13	3	X			3
8	0.12	5	X			2
6	−0.26	3	X			1

*The only animal for which the *DI* increase was due to increased responding to LT −.

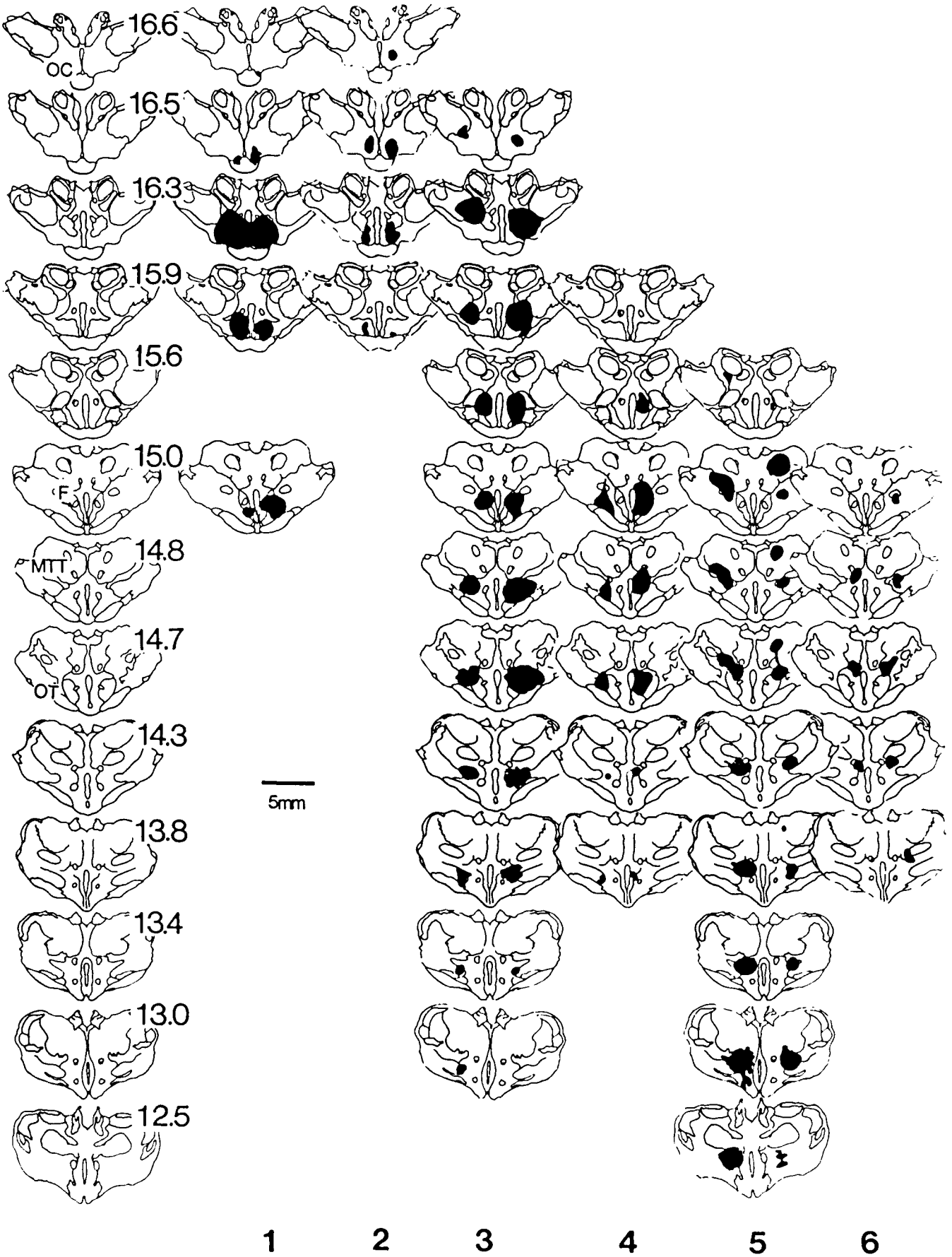

Fig. 1. Reconstruction of lesions for Cases 1–6. The first vertical column of this figure and Figs. 2 and 3 contains representative transverse sections of the rabbit brain from anterior commissure to the red nucleus. Section numbers indicate distances (mm) from an arbitrary zero set at the level of the accessory abducens in histologically prepared tissue. Animal's right is to reader's right. Abbreviations: F, fornix; MTT, mammillothalamic tract; OC, optic chiasm; OT, optic tract.

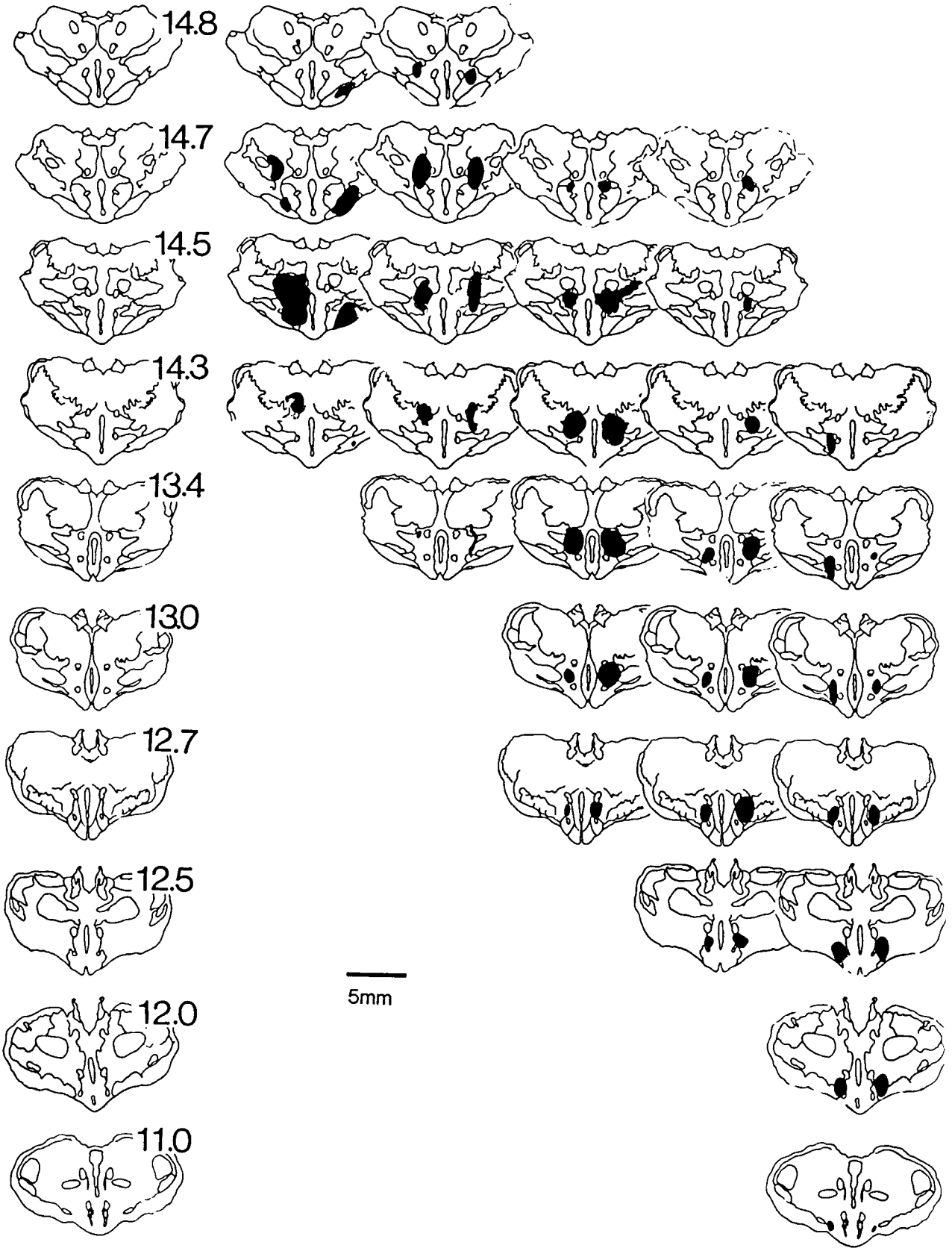

Fig. 2. Reconstructions of lesions for Cases 7–11.

TABLE II

Disrupted animals with mesencephalic lesions

CRT, number of sessions required for criterion. Pre and post designations refer to before and after surgery, respectively. AOA, accessory oculomotor area; HAB, habenular nucleus; PCPT, post-commissural and pretectal area; PAG, periaqueductal gray; RN, red nucleus.

Case	*DI*	*CRT Pre*	*Primary lesion*					*CRT Post*
			PCPT	*PAG*	*AOA*	*HAB*	*RN*	
15L	0.81	3	X					4
12L	0.75	1	X	X		X		2
22R	0.64	6					X	2
20L	0.60	7		X				4
12R	0.57	5	X	X		X		5
19L	0.40	2		X				2
16R	0.35	3		X	X			3
23L	0.33	5		X	X		X	4
20R	0.24	7		X				1

to lowered responding to L+, and the sole exception was not considered disrupted for other reasons (case 11, see below). All animals eventually recovered criterion CI performance (mean number of sessions required to criterion = 2.8, S.E. = 0.49, n = 9).

Table I summarizes the pre- and postoperative CI performance of animals receiving unilateral or bilateral lesions to the hypothalamus. Cases 3, 4, 5 and 7 test the reward system hypothesis of CI. These animals experienced lesions located in the vicinity of the lateral hypothalamus (Fig. 1) and exhibited classic symptoms of the lateral hypothalamic syndrome[5], including aphagia and weight loss. With regard to the reward-system hypothesis stated in the introduction, the lateral hypothalamus has been shown to support self-stimulation in the rabbit[15]. However, for these animals,

TABLE III

Non-disrupted animals with mesencephalic lesions

CRT, number of sessions required for criterion. Pre and post designations refer to before and after surgery, respectively. AOA, accessory oculomotor area; HAB, habenular nucleus, PCPT, post-commissural and pretectal area; PAG, periaqueductal gray; RN, red nucleus.

Case	*DI*	*CRT Pre*	*Primary lesion*					*CRT Post*
			PCPT	*PAG*	*AOA*	*HAB*	*RN*	
13R	0.40*	5	X	X				2
18L	0.33*	4		X				2
25R	0.29*	5		X				2
14R	0.27*	4	X	X				3
21L	0.25*	3		X	X			3
24L	0.16	2		X			X	1
19R	0.14	4		X				1
17L	0.12	1	X	X				1
14L	0.05	1		X				1
13L	0.04	2		X				3
18R	0.04	11	X					1
23R	0.02	5		X				1
24R	0.01	5		X	X			1
25L	0.01	2		X			X	1
22L	−0.06	2		X				2
15R	−0.09	6		X				1
21R	−0.10	10	X	X				2
17R	−0.19	4		X	X			1
16L	−0.28	1		X				4

*Animals for which the positive value of *DI* was due to lowered responding to L+.

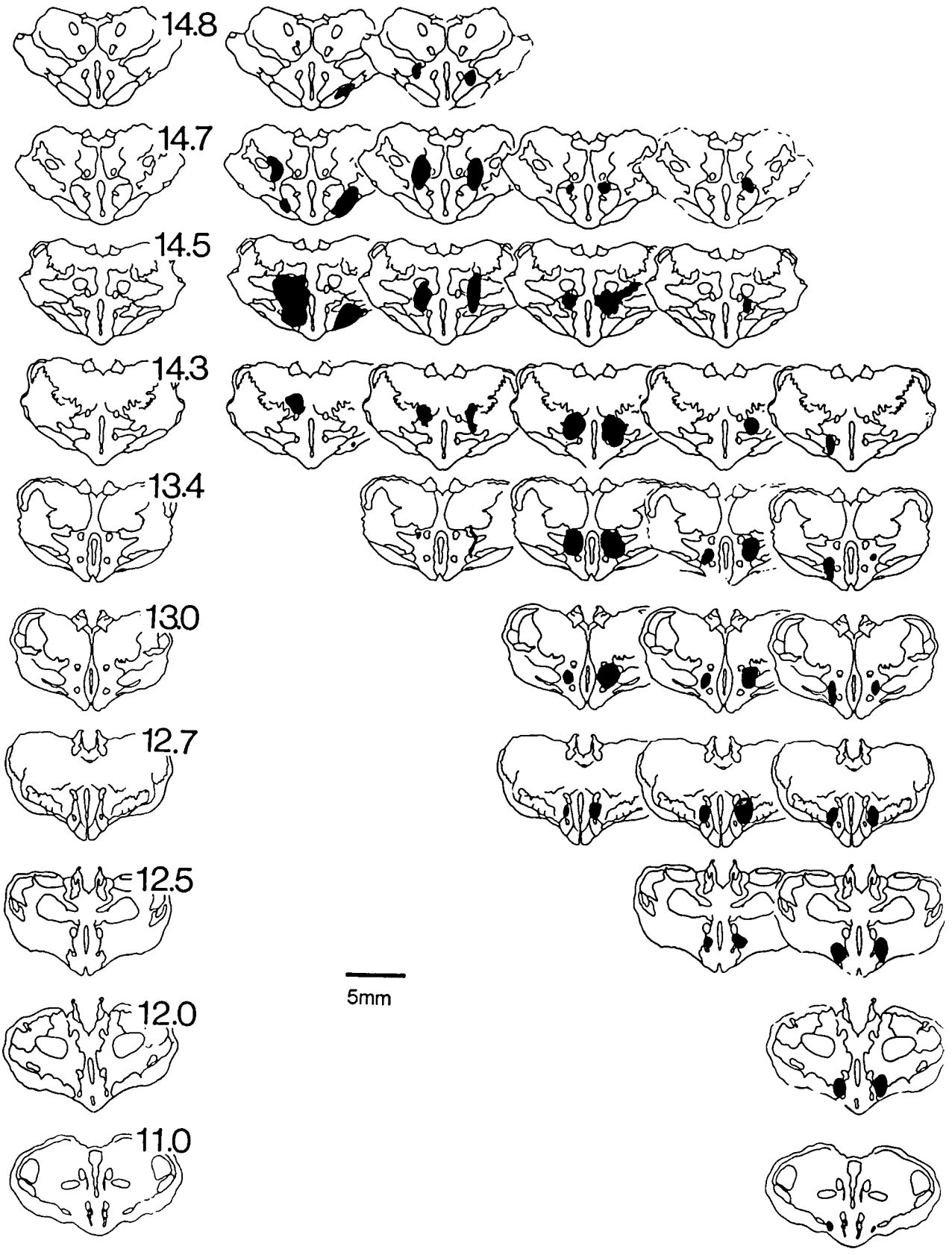

Fig. 2. Reconstructions of lesions for Cases 7–11.

responding to LT – was unaffected (preoperative mean percent CR = 4.0, S.E. = 1.4; postoperative mean = 9.75, S.E. = 4.2) and the changes in *DI* were due to transient decreases in responding to L+ (mean *DI* = 0.23, S.E. = 0.09). This finding supports the conclusion that lateral hypothalamic lesions do not aversively affect classical conditioning[10]. It would appear that they also fail to affect CI. The failure of lateral hypothalamic lesions to increase responding to LT – suggests that the conditioned inhibitor does not serve a rewarding function.

Cases 9, 10, and 11 all had similar bilateral lesions of the posterior hypothalamic region (Fig. 2), and showed a transient postoperative disruption of CI performance. All three animals displayed marked hyper-reactivity, were difficult to restrain, and occasionally emitted vocalizations upon application of wound clips and during the initial presentation of the US. Lesions in all three cases involved the zona incerta to some extent (Fig. 2). Fibers descending from this region (and the caudal hypothalamus in general) have been proposed as comprising part of the nociceptive system[24]. Even well-trained animals will show elevated responding to LT – when aroused or under stress.

CI performance: mesencephalic lesions

Cases 12–25 received mesencephalic lesions following preoperative CI training to both eyes. Tables II and III indicate that left-eye CI training required fewer sessions to attain criterion than the initial right eye training, suggesting a savings effect (left eye mean =

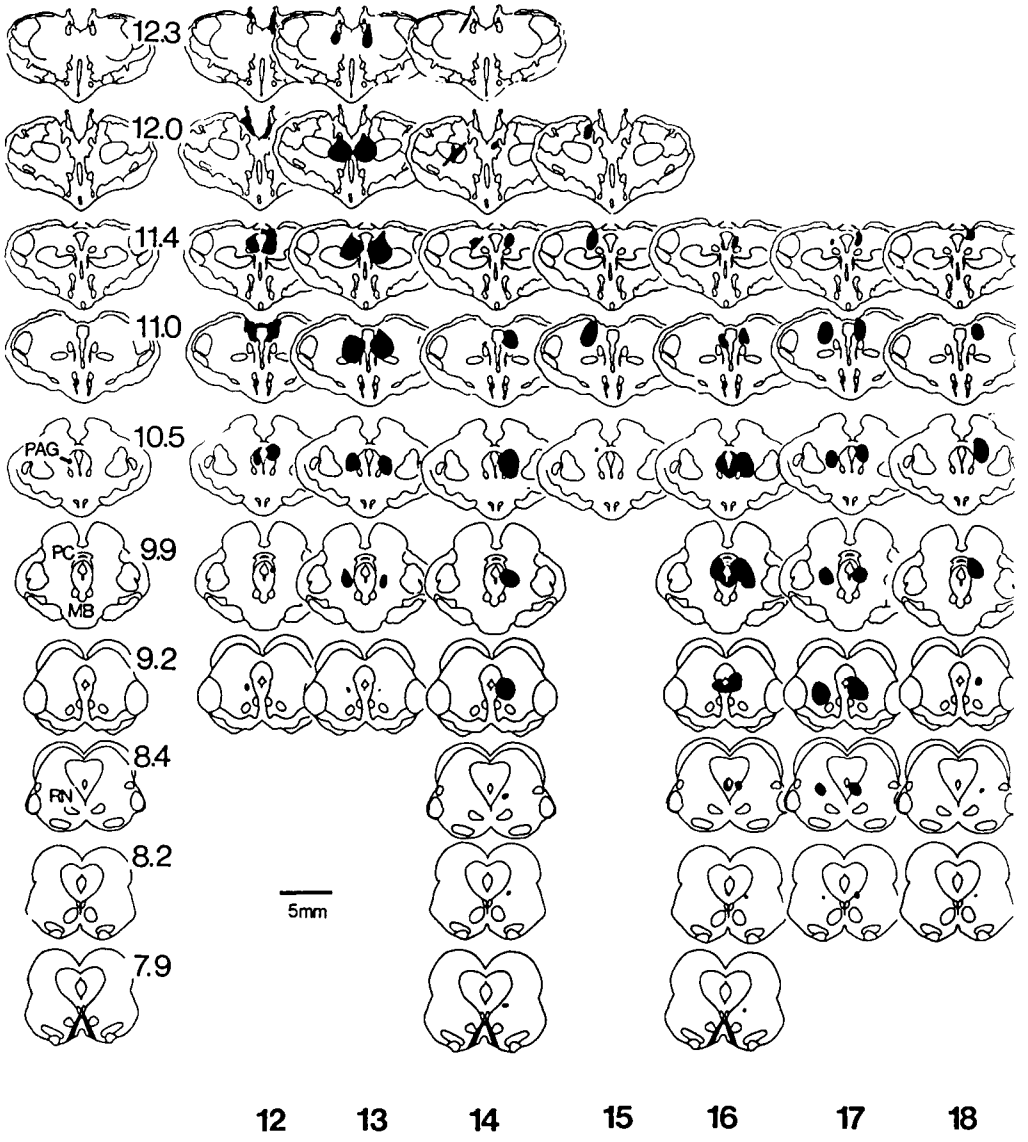

Fig. 3. Reconstructions of lesions for Cases 12–18. Additional abbreviations: MB, mammillary bodies; PAG, periaqueductal gray; PC, posterior commissure; RN, red nucleus.

2.6, S.E. = 0.46; right eye mean = 5.7, S.E. = 0.61, $t(26) = 4.113$, two-tailed $P < 0.001$).

For animals that sustained mesencephalic damage, the overall mean for the *DI*s (for all eyes, $n = 28$) was 0.217 (S.E. = 0.05). Eyes with *DI*s greater than 0.217 that were not due to deflated responding to L + were classified as disrupted (number of eyes = 9, mean *DI* = 0.52, S.E. = 0.07), and the remainder were classified as nondisrupted (number of eyes = 19, mean *DI* = 0.074, S.E. = 0.04). *DI*s for the disrupted eyes were significantly higher than those for non-disrupted eyes ($t(26) = 5.923$, two-tailed $P < 0.001$). As we shall further discuss below, all animals eventually recovered CI performance. For eyes classified as disrupted, the mean number of post-operative training sessions required to re-attain CI criterion was significantly higher than for non-disrupted eyes (Disrupted, mean = 3.0, S.E. = 0.44; Non-disrupted, mean = 1.7, S.E. = 0.21, $t(26) = 2.921$, two-tailed $P < 0.010$).

Table II shows that Cases 12, 15, 16, 19, 20, 22, and 23 exhibited transient CI disruption in at least one eye. Their CI performance returned to preoperative levels

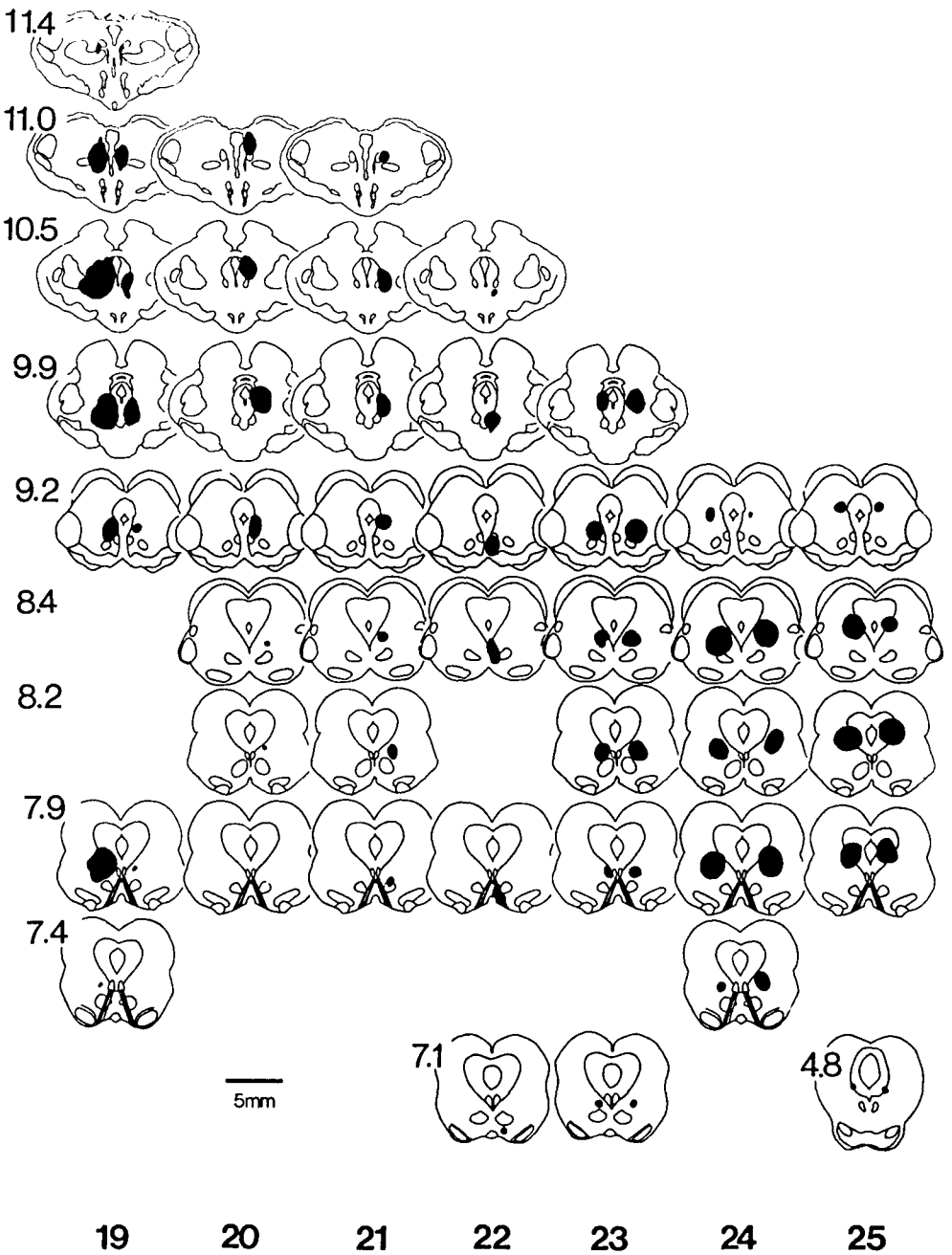

Fig. 4. Reconstructions of lesions for Cases 19–25.

within an average of 2.4 postoperative sessions. Cases with little or no CI disruption are summarized in Table III.

PAG

Damage to the PAG most effectively resulted in CI disruption when lesion placement was fairly rostral (at the level of the posterior commissure, for example) and medial (immediately surrounding the aqueduct). Examples of disrupted cases that showed this pattern include Case 12 (both eyes, Table II, Fig. 3), Case 16 (right eye, Table II, Fig. 3), Case 19 (left eye, Table II, Fig. 4), and Case 23 (left eye, Table II, Fig. 4). In these cases the lesion sites were ipsilateral to the affected eye. However, Case 20 (Table II) showed disrupted CI performance in the left eye despite contralateral damage to the PAG (Fig. 4). Examination of sections falling between sections 11.0 and 11.4 shown in Fig. 4 revealed that the lesion partially encircled the aqueduct. Berthier and Moore[1] also noted contralateral disruption of CI following this type of lesion.

PAG lesions that were caudal to the PC or ventral to the aqueduct produced no disruption of CI. Examples of this pattern include Cases 13 and 14, Cases 17 and 18, the right eye of Case 19, Case 21, Case 24 and 25. The specificity of the placement of PAG lesions is illustrated by cases in which one PAG lesion was more laterally located than the other, resulting in good CI performance in one eye but disrupted performance in the other, for example Case 19 (Tables II and III, Fig. 4).

Pre-commissural/pretectal area

Case 15 (Table II) received a unilateral lesion to the pretectal region (Fig. 3). In agreement with the Berthier and Moore[1] study, this animal displayed a considerable increase in responding to LT −, relative to preoperative performance. This case contrasts with that of the left eye of Case 18, which showed a decrease in responding to L +; the maximal portions of the lesion for Case 18 tended to be more lateral than the disrupting lesion of Case 15 (see section 11.0 of Fig. 3).

Accessory oculomotor area

Case 22 (Table II) received a unilateral lesion to the right accessory oculomotor nuclei and oculomotor nerve (Fig. 4). In agreement with the Berthier and Moore[1] study, this animal showed disrupted CI performance in the right eye. CI performance in the contralateral eye was unaffected by the lesion, and performance in the ipsilateral eye returned to preoperative levels in 2 sessions.

DISCUSSION

Summary

The present study showed that lesions of the lateral and posterior hypothalamus do not affect retention of CI of the NMR. The lateral and posterior hypothalamus are known to support self-stimulation in the rabbit[15] and are therefore considered to be components of a hypothalamic reward system. Under the hypothalamic reward system hypothesis of CI, the conditioned inhibitor serves a reward function because it signals that an aversive event, the US, is not forthcoming[1,7]. The present experiment failed to support the reward system hypothesis of CI, but does not rule out the possibility that the reward system may play a role during the *development* of CI. Such a scenario could be addressed with studies of acquisition of CI following lesions of the lateral and posterior hypothalamus.

The present study also showed that lesions of various mesencephalic structures, including PAG, pretectum, posterior commissure, and accessory oculomotor nuclei, resulted in transient disruption of CI that eventually recovered. The disruption of CI was previously shown by Berthier and Moore[1]. However, these investigators did not observe recovery of disrupted CI, possibly because their use of the Fink–Heimer method allowed for only three days of postoperative retraining. In the present study, recovery of CI performance following disrupting lesions did not become evident until after three postoperative sessions in six of the eight animals judged to have been disrupted.

It is possible that stress or impaired auditory information processing contributed to transient disruptions of CI performance. We have indicated that arousal and stress (e.g. from irritation of periocular tissue) can elevate responding on CS − trials. In the present study, care was taken to mitigate these factors and to take them into account when they occurred. Disruptions of CI in the group with hypothalamic lesions could be accounted for by heightened arousal, as in Cases 9–11. By contrast, CI disruption in the group with mesencephalic lesions, Cases 12, 15, 16, 19, 20, 22, and 23, did not appear to involve these factors. It is unlikely that impaired auditory information processing contributed to CI disruption in the present study. Mis[20] showed that CI-disrupted animals with mesencephalic lesions could acquire a CR to a tone conditioned inhibitor, and in the present study we observed several cases where CI disruption appeared only in one eye. Good CI performance in one eye suggests that the auditory system was functioning normally, since the fibers of lateral lemniscus, the principal auditory pathway in the brain stem, are both crossed and uncrossed[6].

Alternative hypotheses for CI of the NMR

It has been suggested that mesencephalic lesions affect a generalized inhibitory system for the extraocular muscles[20]. The evidence for such a system received some support in the present study because of the transfer of training observed in the animals trained first on the right eye and then on the left (Tables II and III). On the other hand, this construct would have difficulty accounting for the cases in which lesions affected CI performance in only one eye, a situation which parallels lesion data showing that CRs can be disrupted in one eye without affecting either the acquisition or savings in the other eye[25].

Because the conditioned NMR is based on an aversive US, and mindful of reports that stimulation of the pretectal region and the PAG produces analgesia, Moore et al. suggested that the presentation of LT – in this task triggers the release of endogenous opioid peptides[23]. However, naloxone, an opiate antagonist, does not impair CI performance[2].

CI tasks require the active suppression of CRs in the presence of the conditioned inhibitor. Such a selection of behavioral strategy (*not* responding) could be subserved by the diencephalic conduction system[8,30] described by Sutherland[29]. The diencephalic conduction system, situated between limbic forebrain and brainstem regions, includes the septal nuclei, long regarded as candidate structures for the mediation of inhibition, and the habenular nuclei, which have been hypothesized to form a processing station between telencephalon and mesencephalon[26].

Several lines of evidence suggest that the septal and habenular nuclei are involved in CI. Lesions of the septum produced increased responding to the CS – in two-tone differential conditioning of the NMR[16]. Recordings of multiple-unit activity in lateral septum showed an increase in firing during US offset and during presentation of a CS – during aversive conditioning in rat[31]. Neither of these studies were genuine CI tasks because the CS – s were not compounds which included the CS + . Pilot septal lesion studies in our laboratory using the L + /LT – task have yielded ambiguous results because disruptions of CI performance were transient and in some instances manifested themselves as decreased responding to L +, as well as elevated responding to LT – (Blazis, unpublished observations). The possibility that the habenular nuclei might be involved in CI of the NMR is supported in the present study by the disrupted performance of Case 12, an animal that sustained damage to the habenular nucleus. Finally, the septal and habenular nuclei are anatomically well-situated to influence CI via projections through fasciculus retroflexus to the accessory oculomotor nuclei, pretectal region, the PAG, and reticular formation[13,14]. Thus, transections of fasciculus retroflexus could further illuminate the role of the diencephalic conduction system in CI.

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RED NUCLEUS PROJECTIONS TO CEREBELLAR CORTEX (HVI) IN RABBIT EXAMINED WITH WGA-HRP.

M.E. Rosenfield* and J.W. Moore. Dept of Psychol, Univ of Mass, Amherst, MA 01003.

Cerebellar cortex (Larsell's HVI) has been implicated in the generation of the classically conditioned eye blink/nictitating membrane response. The motor program for the conditioned response is presumably learned by the cerebellum and relayed to motoneurons via the red nucleus (e.g., Rosenfield, M. & Moore, J., *Behav Brain Res*, 10:393, 1983). Cat red nucleus reportedly contains neurons that project to cerebellar cortex (Dietrichs, E. & Walberg, F., *Exp Brain Res*, 50:353, *1983*). Projections from the red nucleus to HVI could be important for brain stem and cerebellar processes involved in classical conditioning (e.g., Moore, J. et al, *Biol Cyber*, 62:17, 1989).

We implanted WGA-HRP (Sigma L3892) unilaterally into HVI in 4 albino rabbits (Mori, J., et al, *Brain Res Bull*, 6:19, 1981). The pipette remained *in situ* for 48 ..ours before sacrifice. Animals were perfused transcardially (descending aorta clamped) with approximately 2 L of .9% saline followed by .5 L of 10% formalin and then 3 L of 12% sucrose solution at 4 degrees C. Brains were blocked immediately on extraction (saving only the brain stem and cerebellum), placed in 30% sucrose in .1 M phosphate buff·· (pH = 7.2), and stored at 4 degrees C for 24 h. Brain stem and cerebellum were embedded in gelatin; frozen sections were cut transversely at 60 μ, mounted on subbed slides, and reacted with tetramethylbenzidine. All HVI cases (defined by histological verification of the implantation locus and the presence of retrogradely labeled cells in the pontine nuclei, spinal trigeminal nucleus par oralis, and the dorsal accessory olivary nucleus) showed sparse retrogradely labeled cells in the more caudal (3rd nerve level) portions of the red nucleus, consistent with Dietrich and Walberg's cat study. (Supported by AFOSR grant 89-0391)

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MODULATION OF ACTIVITY IN THE MEDIAL GENICULATE NUCLEUS DURING AUDITORY TRACE CONDITIONING.

K.N. O'Connor and J.W. Moore*. Dept of Psychol, Univ of Mass, Amherst, MA 01003.

In classical trace conditioning the acquisition of a CR is dependent on neural coding of the CS following its termination. There is evidence that the medial or magnocellular region of the medial genicualate nucleus (mMGN) is one area where this coding may occur; it is essential to the acquisition and maintenance of successful discrimination of auditory stimuli in the conditioning of bradycardia in rabbits (Jarrel, et al., *Brain Res*, 382:199, 1986), and multi-unit reponses to reinforced tones in this region increase during conditioning in comparison to non-reinforced tones (Disterhoft & Olds, *J. Neurophys*, 35:665, 1972).

Single cell activity was recorded from the MGN during differential trace conditioning of the rabbit nictitating membrane response (NMR). Rabbits were first trained to discriminate between a reinforced CS+ and nonreinforced CS- (tones of 600 or 1200 Hs) 150 ms in duration followed by a 200 ms trace interval. The US was electrostimulation to the periorbital region of the eye. The rabbits were then prepared for subsequent recording; under anesthesia the dura was exposed, and a recording chamber cemented in place.

Responses of cells in the mMGN tend to be transient and related to the type of CS, primarily showing greater modulation in firing rate to the CS+. Presentation of probe CSs of varying duration suggest a relationship between these responses and NMR timing and topography.

(Supported by AFOSR grant 89-0391 and NSF grant BNS 88-10624)

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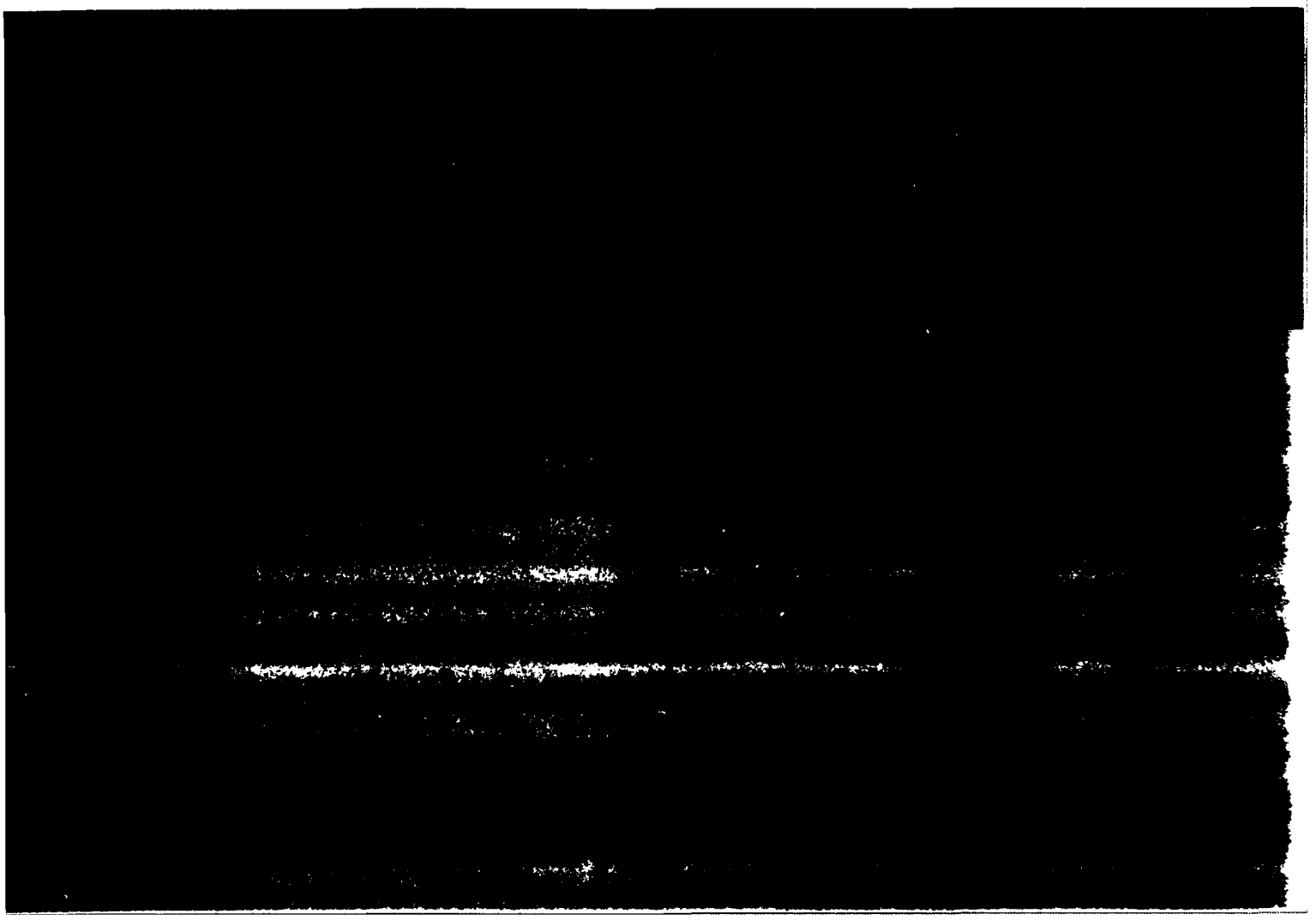

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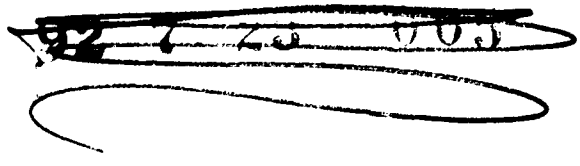

A MECHANISM FOR TIMING CONDITIONED RESPONSES

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ABSTRACT. Classical conditioning procedures instill knowledge about the temporal relationships between conditioned stimuli, which are regarded as predictive signals and triggers for action, and the unconditioned stimulus, the event to be timed. This knowledge is expressed in the temporal features of the conditioned response, which typically develop such that its peak amplitude occurs at times when the unconditioned stimulus is expected. A simple connectionist network, comprised of two neuron-like processing units, provides a mechanism that can acount for virtually all aspects of conditioned response timing. The unfolding of time from the onsets and offsets of events such as conditioned stimuli is represented by the propagation of activity along delay lines. Input to the two processing units from conditioned stimuli arise from collateral taps off of each sequential element of these delay lines.

1. Introduction

Psychologists interested in time, action, and cognition do not typically attend to research on classical conditioning (Block, 1990). One reason for this is that conditioned responses reside in the domain of learning, not cognition and perception. Classical conditioning does not engage the mind—it proceeds beneath the veneer of conscious awareness. Psychologists are interested in impressions and judgements about time. Conditioned responses are time veridical—they reflect real time rather than perceptually distorted impressions of elapsed time. These properties of conditioned responses make them attractive to neuroscientists and computational modellers interested in real-time motor control and its underlying neural mechanisms (Gabriel and Moore, 1990). This community of scholars regards the conditioned response as a microcosm for elucidating behavioral principles of wide generality and for discovering mechanisms for their expression.

This chapter summarizes some facts about the timing of conditioned responses and then presents a neural network model that can accommodate them. From now on, I use the following standard abbreviations: CS for conditioned stimulus, US for unconditioned stimulus, CR for conditioned response, and UR for unconditioned response.

1.1. Conditioning and Cognition

Despite its traditional emphasis on the principles of behavioral learning, it is often convenient to express the outcome of classical conditioning in terms of acquired knowledge.[1] Perhaps the most basic thing learned in classical conditioning is that the CS predicts the US. Prediction is the key to understanding conditioning because a CS's capacity to control behavior depends on the degree to which it is a reliable and nonredundant signal that the US will occur. Subjects learn to ignore stimuli that are poor predictors of the US, and they learn to suppress CRs to stimuli (conditioned inhibitors) that predict the withholding of an otherwise anticipated US.

In addition to expressing knowledge, a CR often possesses the elements of skill. Its topographical features—latency, rise-time, peak amplitude—typically vary from one set of procedures to the next in such a way that they are appropriate to the 'task demands' imposed by training parameters (Levey and Martin, 1968). The main evidence for this adaptive character of the CR is that these topographical features vary systematically with the CS–US interval employed in training. (The CS–US interval is typically abbreviated ISI, for 'interstimulus interval'.) In particular, eye blink CRs are 'temporally adaptive.' Temporal adaptability simply means that the peak amplitudes of CRs occur within a restricted temporal window that also contains the US. In this sense, CRs reflect the knowledge that the US occurs at a specific time after the CS. Recent evidence for this assertion is reviewed later. For now, it suffices to say that subjects learn not only to expect the US in the presence of the CS, but also when to expect it.

1.2. Mechanisms of Knowledge Acquisition and Expression

Classical conditioning procedures establish knowledge about timing. What are the mechanisms that bring this about? What happens in the brain that might explain the temporally adaptive properties of a conditioned eye blink, for example? Learning theorists and neuroscientists alike believe that the knowledge instilled by conditioning, and the accompanying rules for generating CRs, arise from 'associative mechanisms.' These mechanisms are captured by mathematical models that take the form of rules for changing the strength of the 'synaptic connection weights' between representations of the CS and the CR. Such rules consist of two factors—one factor is the level of CS processing; the other factor is the level of US processing (Desmond, 1990; Dickinson and Mackintosh, 1978; Sutton and Barto, 1990; Rescorla, 1988).

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[1]Saying that conditioning procedures produce knowledge does not contradict the earlier statement that CRs arise from unconscious processes (Kihlstrom, 1987).

2. CR Waveforms and CS–US Intervals

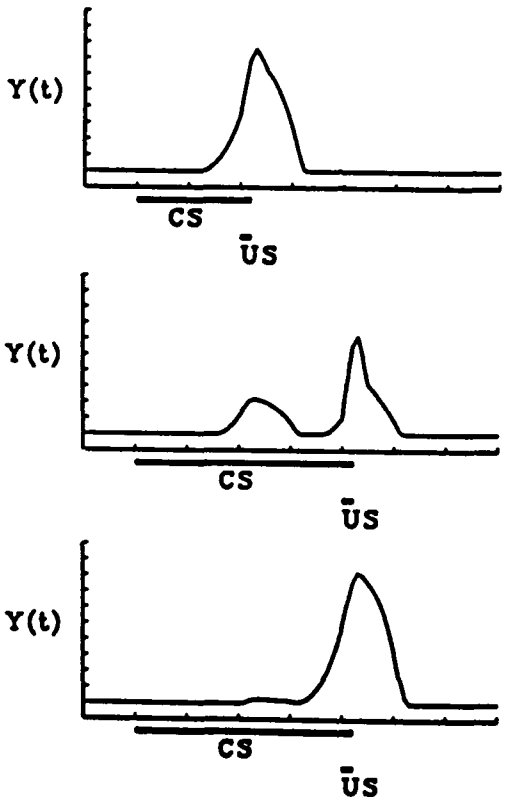

Figure 1. Effects of changing US timing on simulated CR topography, Y(t). Top: Topography after 25 short CS–US interval training trials in Stage 1. Middle: In Stage 2, the CS–US interval is lengthened. After 10 trials, the short-latency CR is somewhat diminished and the longer-latency CR begins to develop. Bottom: After 30 Stage 2 trials, tranformation of CR topography is nearly complete. Copywrite 1989, Springer-Verlag.

Conditioned response waveforms are functions of the CS–US interval used in training. Long CS–US intervals give rise to delayed CRs, which Pavlov attributed to an active inhibitory process which persists until the US is imminent ('inhibition of delay'). There is little evidence to support Pavlov's explanation of the delayed temporal placement of CRs. Instead of being due to inhibition, the phenomenon of delayed CR placement is more likely attributable to the fact that a CR generated by a long CS–US interval is a *different* response from that generated by a shorter CS–US interval.

That a CR generated with one CS–US interval can be regarded as being a different response from one generated with a different CS–US interval is attested to by studies in which subjects are trained with a short CS–US interval and then shifted to a longer CS–US interval. Typically, the CR in the temporal window defined by the short CS–US interval undergoes extinction while a new CR emerges in a window defined by the longer CS–US interval. It is *not* the case that the original CR migrates to the new temporal window. It remains within its original window but progressively loses amplitude while at the same time the new CR emerges in the time window defined by the new US locus. This is illustrated in Figure 1, which is a simulation from the VET neural network model discussed later on.

Additional evidence for the temporal specificity of CRs comes from a widely cited study of rabbit eye blink/nictitating membrane conditioning by Millenson, Kehoe, and Gormezano (1977). Their training protocol consisted of mixing two CS–US intervals, one of 400 msec and another of 700 msec. There were occasional 'probe trials' on which the CS was presented without the US for either 400 or 700 msec. On 400-msec probes, response topography showed a peak appropriate to the 400-msec CS–US interval. On 700-msec probes, response topography revealed two peaks—one appropriate for the shorter interval and another appropriate for the longer interval. This finding implies that subjects learned *If the CS extends beyond 400 msec, initiate another CR; if it does not, do nothing.* However one chooses to phrase it, the implication remains that subjects learned not one CR but two, one appropriate for each CS–US interval it experienced.

2.1. CR Waveforms and Trace Conditioning

The Millenson et al. (1977) experiment used a delay conditioning protocol, which is technical jargon for the fact that, on training trials, CS onset preceded the US and stayed 'on' until the US occurred, at which time it went 'off.' But what of trace conditioning protocols in which CS onset precedes the US but goes off beforehand? Where do subjects place their CRs: during the CS's 'on' phase or during its 'off' phase? As in the case of delay paradigms, CRs occur near the US, so CRs occur during the 'off' phase.

In trace conditioning there are two possible CS–US intervals, one defined by CS onset and the other defined by CS offset. Hence, two things might be learned: the US follows CS onset; the US follows CS offset. Which event, CS onset or offset, defines the temporal window in which to place CRs? The answer is *both*, although this is constrained by such details as the CS's duration and the time between its offset and the US, the so-called trace interval. Support for this conclusion comes from an experiment

by Desmond and Moore (1991a). They trained rabbits using a trace conditioning protocol in which the CS was a 150-msec tone followed 200 msec later by the US, giving a nominal CS–US interval of 350 msec. The US was a mild eye shock, and the CR was extension of the nictitating membrane. A second group of animals were trained in a delay conditioning procedure in which the tone was of 350 msec duration.

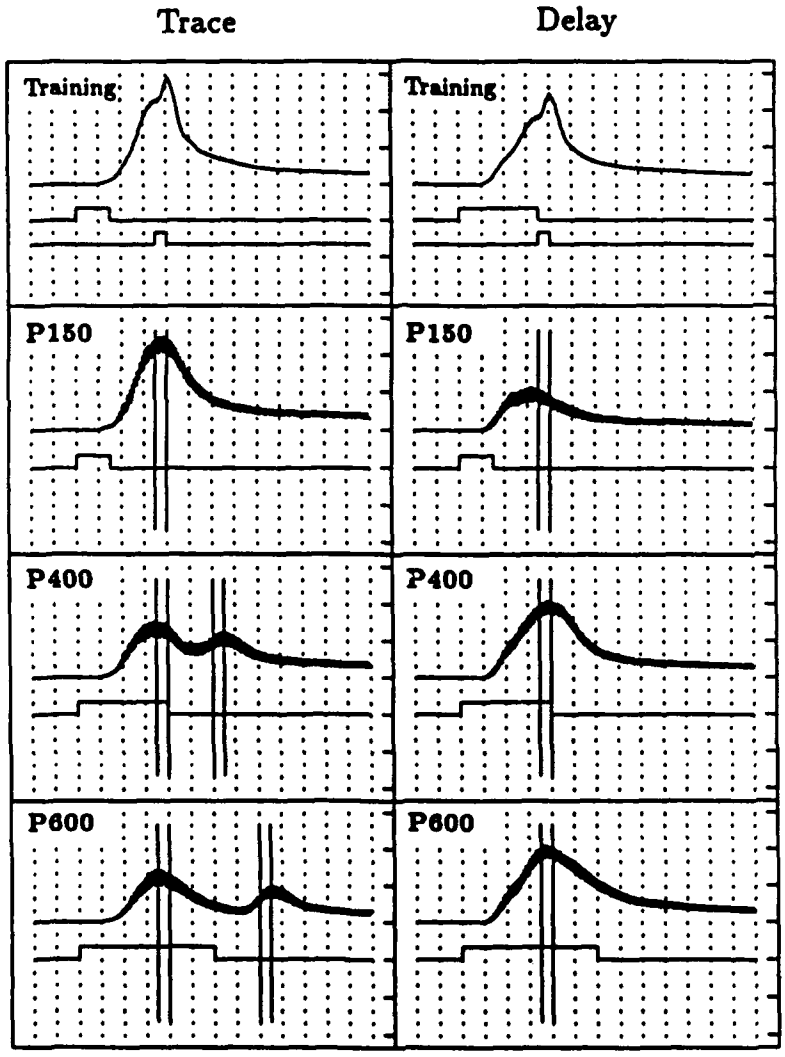

Figure 2. Average CR waveforms from training and probe trials for the Trace and Delay groups of Desmond and Moore (1991a). Fine-grain bars on waveforms during probe trials are standard error bars. Vertical cursors correspond to expected times of the US with respect to CS onset and offset. Copywrite 1991, Springer-Verlag.

After training, subjects in both the Trace and Delay groups were given probe trials without the US. These consisted of presentations of the tone for durations of 150, 400, and 600 msec. As Figure 2 shows, the two longer duration tones often resulted in bimodal (double peaked) CR waveforms in the Trace group, but not in the Delay group. The initial peak was located 350 msec after tone onset, within the temporal window defined by the interval between CS onset and the US. The second peak was located 200 after tone offset, within the temporal window defined by the interval between CS offset and the US. This second peak, though inappropriate for the nominal CS–US interval of 350 msec, is appropriate for the CS–US interval of 200 msec defined in terms of tone offset. Thus, for example, on a typical 600-msec probe trial, one peak appeared 350-msec after tone onset and the other appeared 800 msec after tone onset. Hence, the following knowledge was acquired: The US follows CS onset by 350 msec; The US follows CS offset by 200 msec. Let us turn now to considering how the 'motor program' *Initiate a CR such that peak amplitudes correspond to the times of the US* might be derived from this knowledge.

3. The VET Model

Desmond and Moore (1988) proposed a neural network model capable of simulating the features of CR timing described above (see also, Desmond, 1990; Moore, Desmond, and Berthier, 1989). We refer to this model by the mnemonic VET in order to emphasize its function of mapping associative *values* onto action based on *expectancies* about *timing*. The model assumes that CSs trigger propagated activity in the nervous system. In its simplest form, this activation can be represented by a delay line. A 'tap' or collateral from each element of the delay line encodes the time after the activation has been triggered by the CS. Each potential CS has its own set of delay line elements which are anatomically associated with its modality.[2] In addition to time-tagged stimulus elements, there are two processing units where learning occurs. One unit associates active stimulus elements with the US and passes this information to the other unit, which uses this information to generate appropriate (adaptively timed) CR waveforms. The assumed delay-line representation of time enables these two processing units, which are thought to reside within cerebellar cortex, to treat CS-initiated input as a sequence of discrete events.

In addition to the simulation shown in Figure 1 and the results of the Desmond and Moore (1991a) trace conditioning experiment (Figure 2), the VET model correctly predicts that, as in the case of delay conditioning, CR waveforms in trace conditioning

[2]Desmond (1990) describes an extension of the simple delay line representation of the CS into a planar array. The planar array representation can encompass stimulus generalization and discrimination.

peak at the point of US onset, that is, within the trace interval. The model also predicts that, with long CS–US intervals, CRs do not begin until the US is imminent, the phenomenon that Pavlov attributed to 'inhibition of delay.' Finally, the model predicts the outcome of experiments with multiple CS–US intervals such as the study by Millenson et al. (1977), which showed that training with randomly mixed trials having CS–US intervals of 200 and 700 milliseconds gives rise to CRs with two peaks, each centered at a time of US onset.

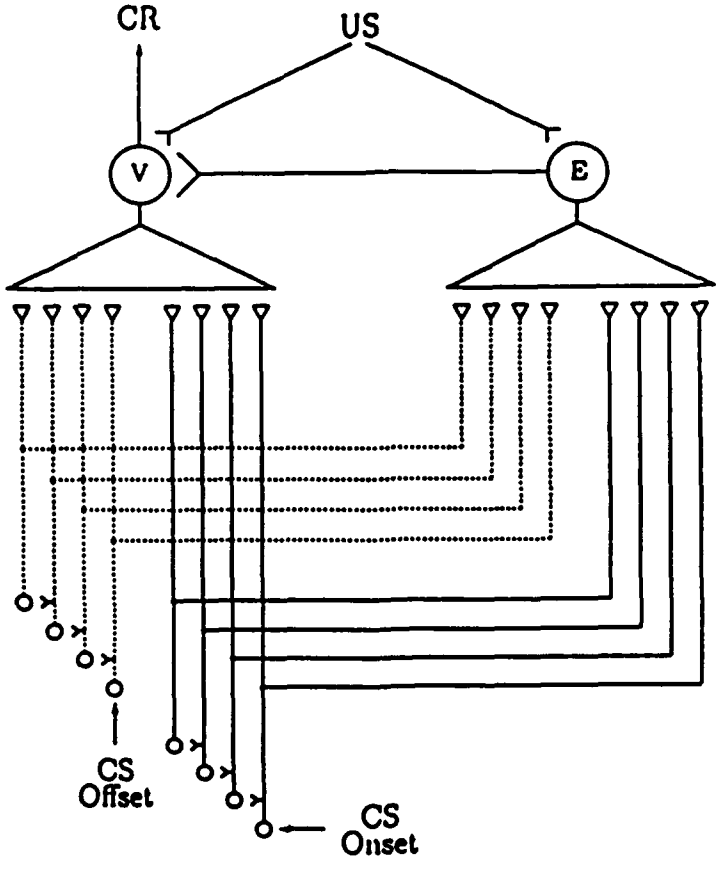

Figure 3. Diagram of the VET network. CS onset and CS offset are assumed to activate separate tapped delay lines that project to the V and E units, as explained in the text. Copywrite 1991, Springer-Verlag.

The structural components of the VET model are depicted in Figure 3. The two neuron-like processing units receive convergent input from CSs and the US. The V-

unit is the output device that generates CR topography. It has modifiable synaptic weights that are changed according to a competitive learning rule. Weight changes depend on local 'eligibility' factors, a global parameter dependent upon the ISI, and a reinforcement signal that reflects the expected time of occurrence of the US. The learning (weight update) rule contains two reinforcement factors: one is contributed directly by the US; the second is contributed by the E unit, which learns when the US occurs with respect to CS onsets and offsets. Both must exceed zero for weight changes on the V unit to occur.

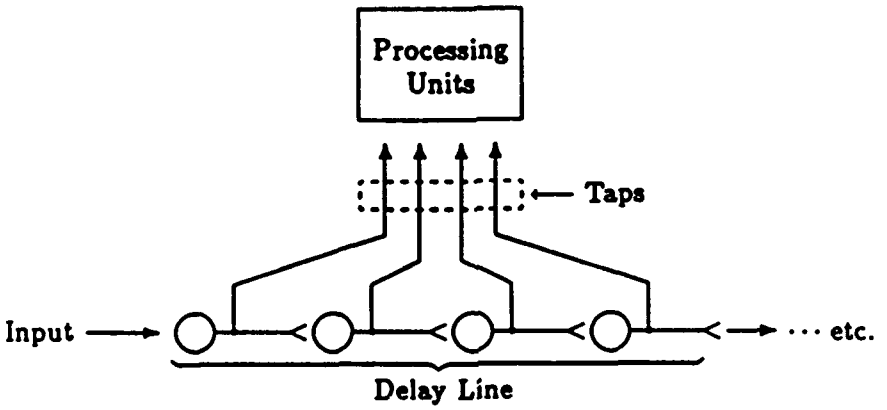

Figure 4. Basic tapped delay line. Injection of CS input begins sequential propagation of signal through a delay line. Each 'synapse' introduces a delay; the total delay from activation of the first element of the delay line to the last element is a direct function of the number of intervening sequential 'synapses' and the conduction speed of propagated activity in the cascade. Taps from the delay line elements send time-tagged information to higher order processing units. Copywrite 1988, Springer-Verlag.

Like the V unit, the E unit receives convergent input from CSs and the US, and it has modifiable synaptic weights that are changed according to a simple linear difference equation that includes local eligibility factors and the global ISI parameter. By providing a precisely timed positive signal to the V unit, the E unit prevents the eventual extinction of positive weights from input elements to the V unit, thereby permitting CR waveforms to anticipate the US. Without this mechanism, the output of the V unit would over the course of training be positive only within time steps that also contain the US.

Conditioned stimuli are provided with a temporal dimension through tapped delay lines (Figure 4) that encode, not only the source of the stimulus (e.g., a particular

component of a compound CS), but also the time since the stimulus began.

Another set of tapped delay lines encodes the time since the stimulus ceased. Thus, the model assumes the existence of separate and independent timed-tagged input elements for both stimulus onset and offset. Figure 5 illustrates how activation of delay line elements are triggered by CS onset and offset.

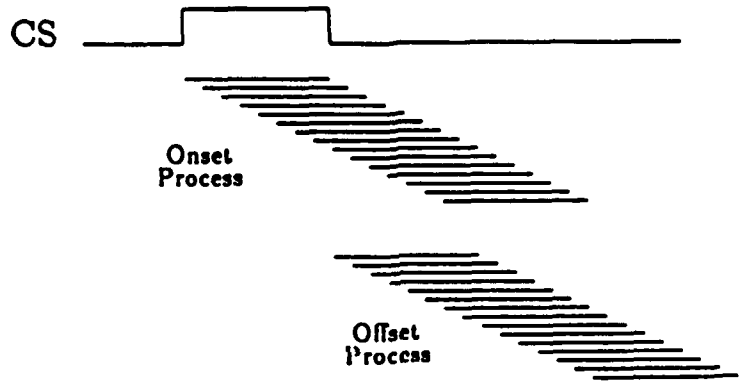

Figure 5. Onset and offset processes for a single CS. Time progresses from left to right. Each horizontal bar below the CS represents the activation time of an individual input element. The figure illustrates the overlapping activation times of individual elements in the onset and offset processes. This feature of the model allows for continous and 'smooth' ramping of the CR from a zero baseline position to its peak amplitude at the expected time of the US, as illustrated in Figures 1 and 2. Copywrite 1988, Springer-Verlag.

4. Brain Implementation of the VET Model

Because of their crucial involvement in eye blink conditioning, we sought to align the VET model with the cerebellum and associated brainstem structures (Moore et al., 1989). We hypothesize that E units, which learn when the US occurs, are Golgi cells and that V units, which are Purkinje cells, use this information to generate an output which ultimately produces a temporally adaptive CR. In brief, Golgi cells learn when USs occur in relation to CS onsets and offsets, and Purkinje cells learns how to generate appropriately timed CRs and their topographical features.

Where does the knowledge that *The CS predicts the US* arise, and what role does it play in the development of adaptively timed CRs? We have suggested that this knowledge comes about through simple Hebbian learning among brainstem neurons.

These neurons provide a 'coarsely coded' version of the CR—one lacking the temporal specificity of the real thing.[3] Their activation by the CS is conveyed to cerebellar cortex where it is fashioned into the appropriate 'finely coded' CR waveform. Specifically, the activation of the brainstem neurons instantiates *The CS predicts the US.* This activation is manifest as a burst of firing that persists for at least the duration of the CS–US interval. When projected to the granule cell layer of the cerebellum, it is intercepted by Golgi cells (E units) at the mossy fiber/granule cell interface.

The Golgi cells have learned to release their normal inhibitory hold on input from the brainstem neurons but only momentarily and at times relative to the CS when the US has occurred in the past. This release of inhibition permits the activation from the brainstem neurons to proceed via parallel fibers to the Purkinje cells (V units) where it can reinforce synaptic modifications of active inputs from the tapped delay line mechanism. In other words, Golgi cells encoding *The US follows the CS by x amount of time* interact with activation arising from the brainstem neurons in such a way as to provide the temporal specificity needed to instruct the Purkinje cells to *Initiate a CR such that the peak amplitude occurs at the time of the US.*

The delay lines illustrated in Figures 3 and 4 are not in the cerebellum but are extrinsic to this structure. Although their location has not been specified or experimentally determined, their existence ought to be evident in the firing of neurons that project to the cerebellum. The most likely place to find such evidence would be in the CS-evoked activity recorded from neurons of the pontine nuclei, which is a major source of mossy fiber input to the cerebellar cortex. This activity would tell us whether information is sent to the cerebellum in the manner implied by Figures 4 and 5. However, it would not inform us about which precerebellar structures are involved in its manifestation—a task that could be approached with fiber-tracing methods.

One candidate for tapped delay lines is the reticular core of the brain, as has been suggested by Scheibel and Scheibel (1967). Reticular formation neurons can fire sustained bursts to a CS (Richards, Ricciardi, and Moore, 1991), they provide a wide range of possible propagation speeds, which are subject to modulation by local and distal processes, and their axons show extensive collateralization, which could provide

[3]Desmond and Moore (1986) reported that the brainstem contains neurons that behave in the manner imagined in this scheme. For example, CS-evoked firing in some supratrigeminal reticular formation neurons predict CR amplitude, across a series of trials, but not CR latency. Some red nucleus neurons behave in this manner (Desmond and Moore, 1991b). By contrast, many cells in the deep cerebellar nuclei show CS-evoked firing patterns that are highly predictive of both CR amplitude and latency (Berthier, Barto, and Moore, 1991).

the taps depicted in Figure 4.

5. Acknowledgement

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